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VOLUME XXV FEBRUARY, 1938 No. 1

STUDIES UPON THE TIME OF FLOWERING OF PLANTS

I. THE RELATION OF NOCTURNAL TRANSLOCATION TO THE TIME OF FLOWERING

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Tolson Memorial Museum, Ravensknowle, Huddersfield, Yorks.

(With 20 Text-figures)

THE work of Garner & Allard (1920, 1923, 1925, 1931) on the response of certain plants to the length of day is, perhaps, one of the most illuminating pieces of botanical research during recent times. Three types of response have been demonstrated, namely, "long-day plants", which flower in the long days of summer, "short-day plants", which remain vegetative during summer and flower in the short days of autumn, and an indifferent type, which flowers at variable periods. It cannot be claimed that a complete explanation of the phenomenon has been advanced, though the original investigators, with C. W. Bacon, performed extensive determinations of hydrogen-ion concentration and water content (Garner *et al.* 1924). An announcement in the original paper (Garner & Allard, 1920), since elaborated by further experiment (Garner & Allard, 1931), seemed to have particular significance; Garner & Allard studied the effect of breaking the continuity of the daily illumination. They found that when plants were placed in the dark for periods of 1–5 hr. in the middle of a long day, the reproductive activities were not markedly changed, as compared with the normal summer illumination. This applied to all short-day plants which were tested, and most long-day plants. Similar periods of darkness, when *continuous* with the natural night, hastened the time of flowering of short-day plants, but seemed in general to have little effect upon long-day plants. Any effect of shortened daylight upon summer-blooming plants was towards the postponement of flowering.

The working hypothesis which prompted the experiments reported in this paper was that a late-flowering or short-day plant did not begin any work of translocation or growth until after a period of several hours in the darkness of night. A long-day plant, on the other hand, would be

likely to commence translocation soon after the advent of night. The possibility of testing this apparent delay in nightly translocation seemed fraught only with the difficulties of a 24 hr. working day. Full collaboration of Mrs M. Grainger, M.Sc., has, however, made it possible to follow the complete daily cycle of metabolism of thirty-four species of plants on ten occasions in 1935 and 1936. Mr G. Sheard, of Leeds University, has contributed material help with some of the determinations.

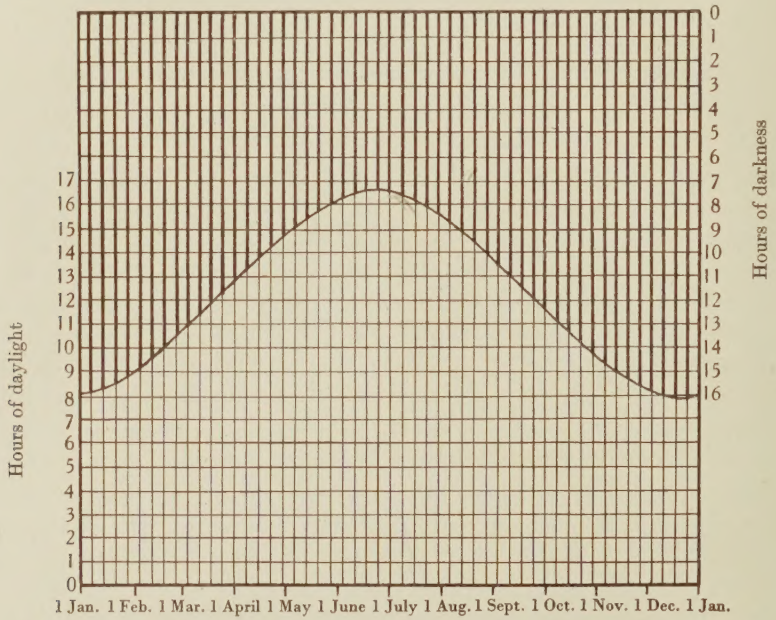

Fig. 1. Duration of daylight (light lines) and darkness (heavy lines) at Lat. 53° 40′ N.

The general plan was to investigate the carbohydrate metabolism of a large number of plant species, using qualitative determinations (section A). Two species were then selected for quantitative determinations, one a typical short-day plant, and the other a long-day species (section B). Several questions which arose as a result of these experiments were further tested by investigations of metabolism in prolonged darkness (section C), and by estimations of the rate of formation of starch when subsequently placed in daylight (section D).

The relative lengths of day and night for any time of the year are set forth in Fig. 1, which refers to the latitude of Huddersfield, 53° 40′ N.

Duration of the period of darkness for particular results is indicated by a thick black line above the diagrams (Figs. 2–14).

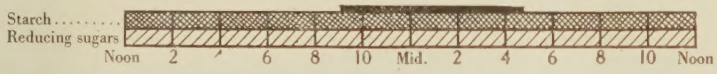

Fig. 2. Wallflower, 8–9 June 1935, vegetative; first-year plants. Similar metabolism was found in: Calceolaria, yellow flowered, 8–9 June 1935, vegetative. *Pteris cretica*, 8–9 June 1935, non-sporing. Stock, 22–23 July 1935, vegetative. *Jasminum nudiflorum*, 24–25 August 1935, vegetative. *Forsythia suspensa*, 24–25 August 1935, vegetative. *Populus canadensis*, 24–25 August 1935, vegetative. Rose—Polyanthus, 24–25 August 1935, flowering. *Tropaeolum*—Scarlet Gleam, 24–25 August 1935, flowering. *Mimulus*, 24–25 August 1935, flowering.

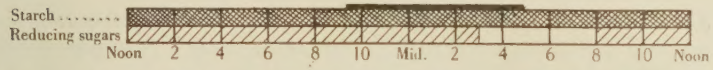

Fig. 3. Early flowering chrysanthemum, 20–21 June 1936, vegetative. Similar metabolism was found in: Antirrhinum, 4–5 May 1935, vegetative; reducing sugars present only from noon to 8 p.m.; 8–9 June 1935, vegetative; reducing sugars present only from noon to 8 p.m. Dahlia, var. Purple Robe, 8–9 June 1935, vegetative; reducing sugar present from 1 a.m. to 4 a.m. *Dianthus Allwoodii*, var. Freddie, 8–9 June 1935, vegetative; reducing sugar present from 1 a.m. to 5 a.m.

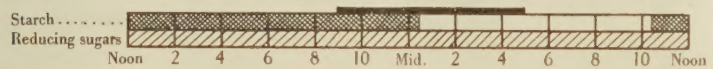

Fig. 4. *Sedum spectabile*, 22–23 July 1935, flowering. Similar metabolism was found in: *Sedum spectabile*, 9–10 April 1936, vegetative. *Reseda*, 22–23 July 1935, flowering; starch absent 3.30 a.m. to 5.30 a.m. *Primula variabilis*, 22–23 July 1935, vegetative; starch absent 10 p.m. to 10 a.m.; 20–21 June 1936, vegetative; starch absent 9 p.m. to midnight. *Primula wanda*, 9–10 April 1936, flowering; starch absent 1 a.m. to 9 a.m. *Vaccinium myrtilus*, 24–25 August 1935, vegetative; starch absent midnight to 6 a.m.

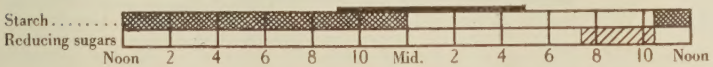

Fig. 5. *Clarkia elegans*, 22–23 July 1935, flowering.

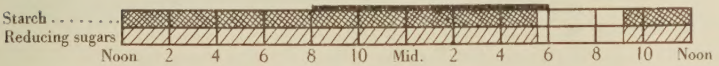

Fig. 6. Lettuce, 24–25 August 1935, vegetative.

Fig. 7. Dandelion, 9–10 April 1936, vegetative.

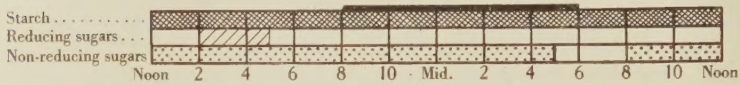

Fig. 8. Laburnum, 24–25 August 1935, vegetative.

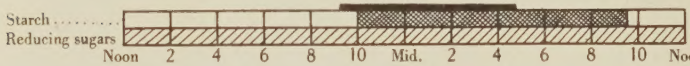

Fig. 9. *Limnanthes Douglasii*, 8–9 June 1935, flowering. Similar metabolism was found in: *Saxifraga tridactylites*, 8–9 June 1935, vegetative; starch present from 10.30 p.m. to 5.30 a.m.; 9 April 1936, flowering; starch present from 7 a.m. to 7 p.m.; 20–21 June 1936, vegetative; starch absent. *Saxifraga Aizoon*, 20–21 June 1936, vegetative; starch absent.

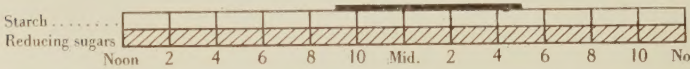

Fig. 10. Gladiolus, 22–23 July 1935, vegetative. Similar metabolism was found in: Auricula, 22–23 July 1935, vegetative, and Daffodil, 9–10 April 1936, flowering.

Fig. 11. *Holcus lanatus*, 24–25 August 1935, flowering. Similar metabolism was found in: *Poa annua*, 24–25 August 1935 and 9–10 April 1936; non-reducing sugars may be present throughout the whole 24 hr.

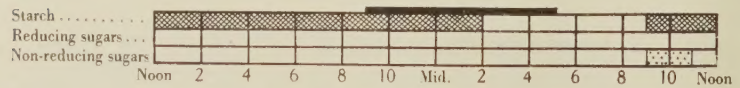

Fig. 12. *Cosmos bipinnatus*, 22–23 July 1935, vegetative.

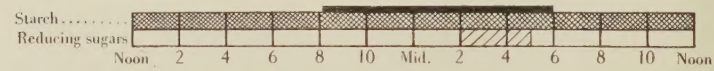

Fig. 13. Soy bean, 24–25 August 1935, vegetative.

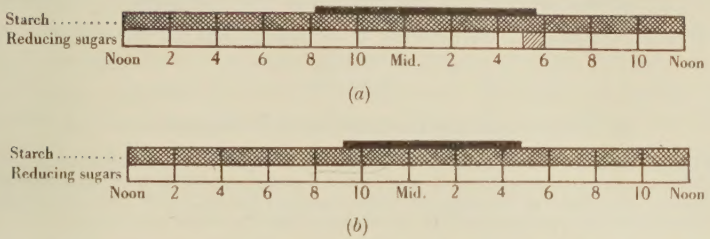

Fig. 14. Late-flowering chrysanthemum, var. Golden Seal: (*a*) in short daylight (long night), 27–28 April 1935; (*b*) in long daylight (short night), 8–9 June 1935.

A. QUALITATIVE DETERMINATIONS

Material and experimental work

A wide variety of plants has been used for qualitative estimations for the presence or absence of starch and sugars. Their times of flowering ranged from February to October, and are set out in Table I. A sufficient number of plants of each species or variety was obtained, so that sample leaves could be taken without significant defoliation. Thus 144 antirrhinum plants were required, whilst fifty chrysanthemums and seventy dahlias sufficed for the experiments. Poplar, jasmine, forsythia, sedum, saxifrages, and some weed species were established in the writer's garden, and usually had sufficient branches or crowns that one or two leaves could be removed for each sample with impunity.

The supply of such numbers of plants would not have been easy, but for the valued co-operation of Miss J. Grainger, of Wilshaw, near Huddersfield, Mr T. Armstrong, Head Gardener of the Ravensknowle Park, Huddersfield, and Mr F. Crawshaw of Marsh, Huddersfield. These helpful gardeners propagated the plants at the appropriate times, and when the seedlings or cuttings were firmly established, they were transferred to the writer's garden at Dalton, Huddersfield, where all further growth took place. No samples were taken until the plants had been in their permanent quarters for at least a fortnight.

Qualitative determinations for the presence of starch were made by the ordinary Sach's test, and for sugars by Fehling's test. Leaves for the latter test were severed and treated immediately. Each was torn into fragments not more than 3 or 4 mm. square. These were introduced into a clean test-tube, and sufficient water to cover them was added. The amount of water in relation to the tissue was therefore roughly standard. This mixture was boiled for 2 min., when the clear liquid was decanted into another test-tube, and the Fehling's test performed for reducing

sugars in the usual way. A separate test for non-reducing sugars was made when required, hydrochloric acid being used for hydrolysis.

Table I

Plants used for the qualitative determinations

Scientific names cited without an authority for the species are interpreted in a horticultural sense. Figures in column 3 refer to the text-figures, which portray results of the experiments. The times of flowering are those observed locally, under the same conditions of climate and exposure as applied to the experiments reported in this paper.

Plant	Variety	Text-fig.	Time of flowering
Antirrhinum majus	Flame	3	Late July
Auricula, *Primula Auricula*	Chocolate-flowered	10	Mid April
Bilberry, *Vaccinium myrtilus*	—	4	May
Calceolaria rugosa	Bronze-flowered	2	Late July
Chrysanthemum, early-flowering	Sunburst	3	Late July
Chrysanthemum, late-flowering	Golden Seal	14	October
Clarkia elegans	—	5	July
Cosmos bipinnatus	—	12	Late July and August
Daffodil	Spring Glory	10	Mid April
Dahlia	Purple Robe	3	July
Dandelion, *Taraxacum officinale* Wigg.	—	7	May
Dianthus Allwoodii	Freddie	3	First week in July
Forsythia suspensa	—	2	February
Gladiolus	—	10	September
Holcus lanatus L.	—	11	August
Jasminum nudiflorum	—	2	February
Laburnum vulgare	—	8	June
Lettuce, *Lactuca sativa*	—	6	July and August
Limnanthes Douglasii	—	9	Early June
Mignonette, *Reseda odorata*	—	4	July
Mimulus luteus	—	2	August
Nasturtium, *Tropaeolum majus*	Scarlet Gleam	2	July and August
Poa annua L.	—	11	Spring, summer and autumn
Populus canadensis	—	2	—
Primula variabilis	—	4	May
Primula wanda	—	4	April
Pteris cretica	—	2	Spored mid-July, but may spore almost continuously
Rose	Polyanthus	2	August
Saxifraga Aizoon	—	9	April
Saxifraga tridactylites	—	9	May and early June
Sedum spectabile	—	4	End of July
Soy bean, *Soya max*	—	13	Late September
Stock, *Matthiola* sp.	Summer-flowering	2	End of July
Wallflower, *Cheiranthus cheiri*	—	2	May and June

The method of extraction cannot be regarded as removing anything but the soluble matter which was free to move in the veins of the tissue, or which was leached out of damaged cells. Such a short period of boiling could not extract the soluble matter from within the cells of the relatively large pieces of leaf. These simple methods yield results of comparative value by virtue of the positive insight which they give into the nightly metabolism.

Samples were taken, and determinations made, approximately every 3 hr., throughout a period of 24 hr. The times of sampling were not always the same, but in the accompanying diagrams (Figs. 2–14 and 18–20) any change recorded thereon is represented arbitrarily as taking place midway between the times of the preceding and succeeding determinations. Thus in Fig. 3, for example, the time of disappearance of reducing sugar, namely 3 a.m., is inferred from a positive result of a Fehling's test at 1.30 a.m. and a negative result at 4.30 a.m. The very narrow band of reducing sugar in Fig. 14*a* is due to the fact that determinations were made at less than 3 hr. intervals in this particular case. The adoption of this arbitrary device should render the portrayal of the results by the figures sufficiently accurate, without the use of extensive tables.

Results

The diagrams illustrating the metabolism of long-day plants (Figs. 2–11) show that all species have potentially mobile carbohydrates. Reducing sugars are very soluble and easily transportable, and they are usually present all the time (Figs. 2, 4, 9, 10) or during a considerable part of the 24 hr. period (Figs. 3, 6, 7). Starch often disappears during the night (Figs. 4, 5, 6, 7), thus showing that there is a change from this insoluble carbohydrate, to a soluble and more transportable sugar. A few plants, e.g. *Holcus lanatus*, *Poa annua* and laburnum (Figs. 8, 11) show a diurnal periodicity of non-reducing sugar, which must be the transportable carbohydrate of these plants, instead of reducing sugar.

The short-day plants (Figs. 13, 14) are in marked contrast, for they have relatively immobile carbohydrates. No disappearance of starch can be shown, and in the short nights of summer no reducing sugar can be detected qualitatively (Fig. 14*b*). Only in the relatively long nights of April or late August has the presence of reducing sugar been detected immediately before dawn (Figs. 13, 14*a*). No periodicity of non-reducing sugar could be detected in either the late-flowering chrysanthemum or the soy bean.

This evidence upholds the working hypothesis mentioned above, that true late-flowering or short-day plants do not begin the work of translocation until several hours after the fall of darkness. Even in long nights, the production of a transportable sugar does not begin until a short time before dawn (Fig. 14*a*), and in the short nights of summer it may not occur upon a measurable scale. Starch formed in one period of sunlight has not been transported when the sun's rays again visit the earth. The amount of carbohydrate which is available for growth

activities is therefore limited, in comparison with long-day plants. Short-day plants are, relatively, starving for sugar in the midst of an abundance of immobile carbohydrate.

The attainment of a suitable ratio of carbohydrate to nitrogen has been advanced by Kraus & Kraybill (1918) and others as a cause of flowering, but the discovery of photoperiodic response by Garner & Allard seemed to indicate the presence of another factor. It would appear from the present results that a delay in the translocation of carbohydrate in short-day plants could delay flowering by virtue of its effect upon the carbohydrate-nitrogen ratio. The plant's supply of nitrogen from soil sources is probably maintained steadily throughout the summer, and is not likely to depend much upon relative length of day. Thus long-day and short-day plants would seem to have the same facilities for nitrogen intake, but the summer-blooming plants can put their carbohydrates into circulation, where short-day species have stores of unavailable starch and sugars—a "frozen currency" in the carbohydrate sense. A suitable carbohydrate-nitrogen ratio would therefore be attained quickly by long-day plants, and slowly by the short-day kinds, hence the delay in flowering of the latter.

It seemed that a possible explanation of the earlier flowering of short-day plants when summer nights are lengthened artificially, as mentioned above, might be found in the increased time for translocation afforded by the extended period of darkness. Experiments were designed to test this hypothesis, and are described in section C of this paper.

The number of early-flowering and summer-blooming plants used in these experiments is very much larger than that of late-flowering plants. Two only of the latter have been found with certainty, namely, late-flowering chrysanthemum and soy bean. A third species, *Cosmos bipinnatus*, was enumerated as a short-day plant by Garner & Allard, but its flowering period in Yorkshire often begins as early as the end of July. Other plants, raised from the same seed and grown in the same garden, may bloom as late as the end of September. Though it may be made to flower earlier by curtailing the length of day in summer, it is not such a typical short-day plant as the late-flowering chrysanthemum. The diagram of its carbohydrate metabolism (Fig. 12) also shows a disappearance of starch, and a diurnal periodicity of non-reducing sugar content. This is more typical of a long-day plant than a short-day species.

A number of plants flowering in the relatively short days of the early part of the year seem to have similar metabolism to the more typical long-day plants. Such are forsythia, jasmine, the primulas, daffodil,

auricula and the saxifrages. Most of these plants have some peculiarity of structure or of organization to account for their early blooming. The daffodil has a very specialized periodicity closely bound with its bulb habit and its monocotyledonous organization. Forsythia and jasmine are shrubs whose flower buds are made in the previous year; the primulas, auricula and the saxifrages have a similar habit. The saxifrages present an interesting case. Indications have been gleaned from the present experiments that these plants, whilst having in general a long-day metabolism, have a different detail of diurnal periodicity in the spring, from what appears in summer. This question, and that of climate in relation to daily metabolism, will be discussed in a separate paper.

B. Quantitative determinations

The cruder qualitative determinations must give place to more accurate estimations, once they have signified agreement with the working hypothesis. Experiments recorded above show that the early-flowering and late-flowering chrysanthemums would provide suitable material for comparison of the metabolism of long-day and short-day plants respectively. These species are similar, but the difference between their times of flowering is considerable, namely, between late July and early October. Samples were taken at approximately 3 hr. intervals during a substantial part of a 24 hr. period. Many more plants were, of course, needed than for qualitative work. Whole leaves were pulled from the shoot, and eight of them, taken from corresponding positions upon different plants, usually constituted the sample.

The fresh weight was obtained, each group of leaves was placed in a paper tray, and was then heated within a steam oven for 3 hr. All samples were removed, at the expiration of the 24 hr. period, to a drying oven at 65° C., where all remaining moisture was driven off. The dry weight was then obtained, the leaves were powdered in a mortar, and stored in glass tubes placed within a desiccator.

About 0·05–0·1 g. of the powdered tissue was weighed for analysis into a 3×1 in. glass tube. Alcohol (95 %) was then added at the rate of 10 c.c. per tenth of a gram of the sample, and the cold mixture was left for at least 36 hr. This method of extraction is very different from that employed in the qualitative work; it extracts all soluble matter, where simply boiling with water for 2 min. would only remove any soluble matter which was free to move in the leaf, or which was leached out from damaged cells.

The insoluble residue was filtered off with frequent washing, dried at 65° C., weighed, and then boiled with 3% sulphuric acid for 3 hr. It was then neutralized with sodium carbonate, filtered, washed with distilled water, and the filtrate made up to standard volume. Estimations on this liquid gave the content of insoluble carbohydrate. The alcoholic filtrate was made up to standard volume with the requisite quantity of 95% alcohol, and was estimated for soluble reducing sugars, and, when required, for non-reducing sugars.

Table II

Early-flowering chrysanthemum, 20–21 *June* 1936.
Sunset 9.20 *p.m.* *Sunrise* 4.42 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Figs. 15, 16.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
2.30 p.m.	19·20	84·70	51·27 ± 1·02	Trace	10·25 ± 0·508	40·34 ± 1·18
7.30 p.m.	20·90	80·61	58·38 ± 1·40	Trace	13·01 ± 0·645	44·94 ± 1·61
10.30 p.m.	19·35	74·92	45·14 ± 0·571	2·07	9·07 ± 0·571	34·00 ± 1·57
1.30 a.m.	19·18	64·22	43·86 ± 0·855	Trace	15·57 ± 0·857	28·00 ± 0·571
4.30 a.m.	18·25	71·25	37·02 ± 0·693	1·09	11·48 ± 0·594	24·55 ± 1·40
8.00 a.m.	18·29	67·90	32·56 ± 0·660	5·29	8·11 ± 0·377	19·06 ± 1·42
			Old leaves			
2.30 p.m.	16·33	74·42	32·56 ± 0·425	2·70	13·33 ± 0·283	16·53 ± 0·425
7.30 p.m.	13·24	63·06	32·31 ± 0·615	Trace	12·61 ± 0·615	18·77 ± 1·08
10.30 p.m.	14·65	74·61	40·16 ± 0·390	5·07	7·92 ± 0·130	27·17 ± 0·974
1.30 a.m.	11·54	77·00	42·60 ± 0·800	7·00	8·60 ± 0·400	27·00 ± 0·800
4.30 a.m.	12·24	75·00	39·42 ± 0·750	Trace	13·00 ± 0·750	25·55 ± 0·750
8.00 a.m.	12·99	72·45	37·68 ± 0·870	Trace	16·81 ± 0·870	20·00 ± 0·290

The picrate method of Willaman & Davidson (1924) was used to estimate the carbohydrates. This is convenient and accurate for comparison. It was found that the method gave reliable readings for total carbohydrates, and for reducing sugars, but discordant results were sometimes obtained for soluble non-reducing sugars. This would seem to be occasioned by the use of the picric acid as a hydrolysing agent, as recommended by Willaman & Davidson (1924). It has been mentioned by Davis *et al.* (1916) that certain hydrolysing acids destroy the fructose resulting from hydrolysis, and citric acid has been mentioned as an acid safe in this respect. Citric acid (10 and 20%), however, inhibits the

Table III

Late-flowering chrysanthemum, 20–21 *June* 1936.

Sunset 9.20 *p.m. Sunrise* 4.42 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Figs. 15–17.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
2.30 p.m.	16·20	78·78	31·67 ±0·444	3·53	9·66 ±0·303	18·48 ±1·06
7.30 p.m.	15·42	75·71	30·85 ±0·714	2·80	9·35 ±0·322	18·70 ±0·807
10.30 p.m.	15·89	82·77	31·18 ±0·515	Trace	10·13 ±0·267	20·19 ±0·533
1.30 a.m.	16·06	74·62	33·05 ±0·833	5·85	9·23 ±0·380	17·97 ±0·759
4.30 a.m.	14·86	79·98	32·05 ±0·972	6·90	7·68 ±0·316	17·47 ±0·526
8.00 a.m.	15·68	76·89	26·24 ±1·64	Trace	9·35 ±0·385	16·54 ±0·513
			Old leaves			
2.30 p.m.	13·31	84·85	29·05 ±0·625	2·56	8·65 ±0·471	17·64 ±0·471
7.30 p.m.	12·60	70·25	32·00 ±0·500	9·23	8·35 ±0·329	14·42 ±0·549
10.30 p.m.	13·72	68·02	34·65 ±1·16	6·21	13·88 ±0·695	14·58 ±0·695
1.30 a.m.	12·01	74·92	28·22 ±0·444	1·35	11·87 ±0·625	15·00 ±0·625
4.30 a.m.	12·29	74·20	34·43 ±1·47	4·74	14·54 ±0·605	15·15 ±0·605
8.00 a.m.	12·58	59·50	31·02 ±0·821	8·18	9·05 ±0·357	13·79 ±0·357

Table IV

Late-flowering chrysanthemum, 9–10 *April* 1936.

Sunset 6.45 *p.m. Sunrise* 5.20 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Fig. 17.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
6.30 p.m.	9·75	59·16	26·83 ±0·500	12·49	10·17 ±0·333	4·17 ±0·830
10.30 p.m.	9·15	59·23	23·30 ±0·539	8·31	10·92 ±0·385	4·07 ±0·769
2.30 a.m.	7·25	70·42	23·31 ±0·352	7·61	6·90 ±0·143	8·80 ±1·41
5.30 a.m.	8·34	62·22	16·22 ±0·555	4·22	5·89 ±0·222	5·11 ±1·11
8.30 a.m.	9·20	55·74	17·40 ±0·261	6·18	9·04 ±0·174	2·18 ±0·870
			Immature leaves			
6.30 p.m.	12·61	73·57	27·29 ±0·500	4·15	6·28 ±0·286	16·86 ±0·715
10.30 p.m.	12·10	73·04	14·63 ±0·522	3·65	8·78 ±0·267	2·30 ±0·609
2.30 a.m.	11·00	58·20	18·25 ±0·246	4·10	10·98 ±0·164	3·17 ±0·574
5.30 a.m.	10·87	68·75	12·95 ±0·535	4·11	7·05 ±0·268	1·79 ±0·446
8.30 a.m.	12·02	63·87	23·32 ±0·323	6·20	9·03 ±0·645	7·99 ±0·645

formation of the brown colour typical of picramic acid, and therefore nullifies the test. The chrysanthemum material seemed to respond quantitatively to hydrolysis by 3 % sulphuric acid, as mentioned above, so the total carbohydrate was estimated by separate hydrolysis of an unextracted sample of the original powdered tissue. The figures under the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" in Tables II, III and IV have therefore been estimated by direct analysis, whilst the figures in the column headed "Non-reducing sugars" have been obtained by difference.

Results

Tables II–IV and Figs. 15, 16 show that there is a delay in the translocation of carbohydrates from fully expanded leaves of late-

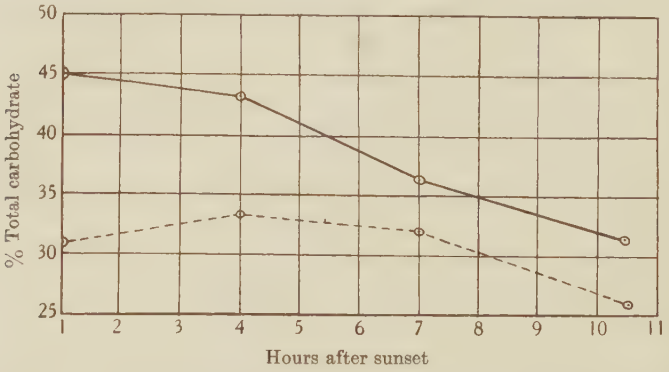

Fig. 15. Total carbohydrate content during the night of 20–21 June, 1936 (long-day conditions). Note the sustained and considerable reduction (13 %) in the early-flowering chrysanthemum (——), compared with the slight (5 %) and delayed reduction in the late-flowering kind (– – –).

flowering chrysanthemums, as compared with the early-flowering kind. It is not shown, however, by an increase in the amount of soluble reducing sugar as in the qualitative determinations, but by a decrease in the total amount of carbohydrate during the night. This is a surer criterion, and confirms the findings of the qualitative experiments, but from a different angle. Old leaves were also estimated for comparison, and showed that no substantial decrease in total carbohydrate took place during the night, in both early- and late-flowering chrysanthemums. What use are these old leaves to the plant? They transpire large quantities of water, their photosynthetic activity is at a minimum, and

they seem to act as unnecessary reservoirs for the storage of carbohydrates which could make a far greater contribution to the plant's growth if they were delivered at the growing-point. Young leaves, not more than 15 mm. long, showed a variable metabolism (Table V). It is interesting to note that leaves of the early-flowering chrysanthemum contain more carbohydrate at all times than the late-flowering kind.

Comparison of the behaviour of the late-flowering chrysanthemum in long-day illumination (Table III) with that under short-day conditions (Table IV) shows the relation of this delayed translocation to the relative length of night. Fig. 17 illustrates such a comparison; in June daylight appears before the fall assumes any substantial proportions; in April the

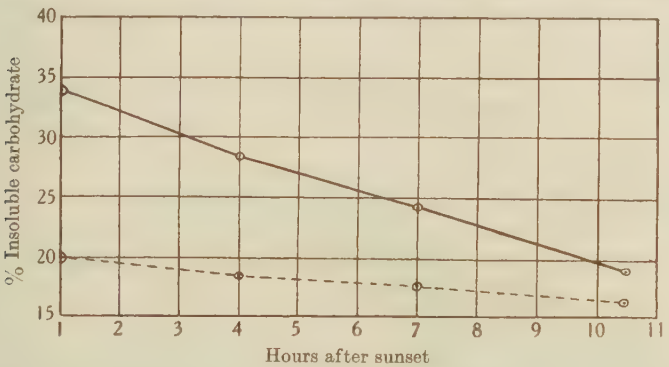

Fig. 16. Insoluble carbohydrate content during the night of 20–21 June 1936 (long-day conditions). Note the considerable reduction (15%) in the early-flowering chrysanthemum (——), as compared with the slight reduction (4%) in the late-flowering variety (– – –).

fall occurs about 3 hr. before dawn. It is not to be expected that the content of total carbohydrate would rise immediately after dawn; there is a time lag before it begins to rise. A chrysanthemum plant takes a few hours to manufacture starch, even when previously kept in the dark, as is shown later in this paper. The lag follows a prolonged period of translocation in the short-day conditions, and only a short one during the long days of summer.

Table IV shows that no large increase in the amount of reducing sugar appears immediately before dawn, in April, in the late-flowering chrysanthemum, as would be suggested by the qualitative tests (Fig. 14*a*). This would be accounted for by the difference in methods of extraction, and the most likely inference is that the cells of late-flowering chrysanthemum leaves become more permeable a little time before dawn. They

would thereby allow more reducing sugar to leach out into the water used for extraction in the qualitative determinations. Extraction of *all* the soluble constituents by alcohol would not necessarily make any such discrimination.

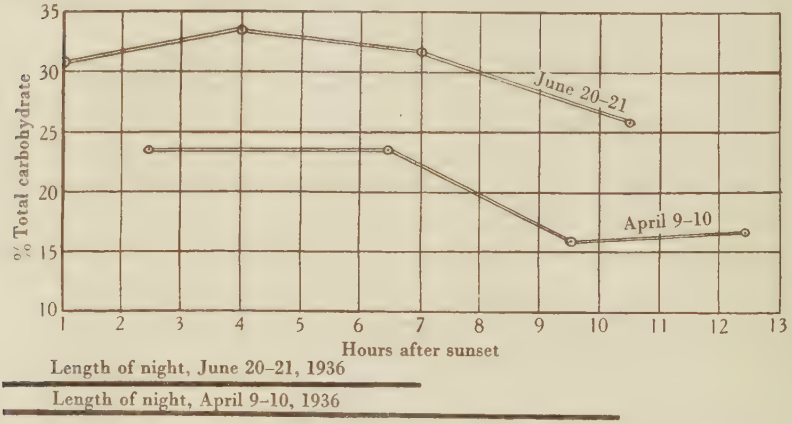

Fig. 17. Comparison of the total carbohydrate content of the late-flowering chrysanthemum, under long-day conditions (20–21 June) and short-day conditions (9–10 April).

C. Experiments with prolonged darkness

A repetition of Garner & Allard's original experiment upon the hastening of flowering of short-day plants by artificially lengthening the nights in summer was made at Weetwood, Leeds, in 1932. Late-flowering chrysanthemums of the varieties Golden Seal, Rayonanthe, Mrs N. Wells, Thora and Mauve Single, were raised from cuttings, and were grown in 10 in. pots in the usual way. They were subjected to a 12 hr. day, from 7 a.m. to 7 p.m. from 13 July, and by 25 August all varieties had bursting flower buds. Control plants, grown in normal daylight, did not flower until early October.

The qualitative experiments of section A show that reducing sugars can only be detected in the late-flowering chrysanthemum immediately before dawn, even in a relatively long night (Figs. 14*a*, *b*), and there is the above experimental evidence to show that flowering is hastened by lengthening the night artificially. Can reducing sugars be detected for a longer period during a night artificially lengthened? The following experiments suggest an answer in the affirmative.

Numerous plants of the late-flowering chrysanthemum, Golden Seal, were taken from the cool greenhouse of the writer's garden, and were

placed in a well-ventilated dark shed, in the early evening of 27 April 1935. Samples were tested for starch and reducing sugars at approximately 3 hr. intervals, until 4 p.m. of the following day. The results are given in Fig. 20, which shows that reducing sugars were present in detectable amounts for several hours. We now have the following facts:

(1) Late-flowering chrysanthemums, representing typical short-day plants, exhibit delayed translocation of carbohydrate through the night. During the long nights of April reducing sugar can be detected qualitatively only for a short time before dawn, and in the short nights of

Fig. 18. Wallflower in prolonged darkness, 8–9 June 1935. Similar metabolism was found 8–9 June 1935 in: Calceolaria, Antirrhinum and Dahlia; but, in the two latter, reducing sugar did not persist long after the beginning of darkness.

Fig. 19. *Limnanthes Douglasii* in prolonged darkness, 8–9 June 1935. Similar metabolism was found in *Saxifraga tridactylites*, 8–9 June 1935.

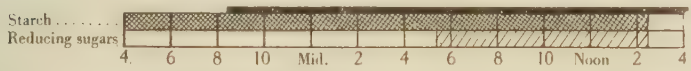

Fig. 20. Late-flowering chrysanthemum in prolonged darkness 8–9 June 1935.

summer it cannot be found at any time during a 24 hr. period. Such plants bloom naturally in the short days of late September or October.

(2) An artificial lengthening of the night in summer causes the reducing sugars to be detectable for a longer period, and at the same time makes the late-flowering chrysanthemum to flower in the relatively long days of August.

(3) Normal summer-blooming or long-day plants have reducing sugars detectable qualitatively for a considerable time during the 24 hr. period, or exhibit other evidence of the mobility of their carbohydrates.

The logical conclusion seems to be that an artificially lengthened night during summer causes a short-day plant to flower earlier than normal by virtue of the extended time for transportation of carbohydrate which it confers. This artificial treatment renders both the carbohydrate

metabolism, and the time of flowering, more like those of a long-day plant. A comparison of Figs. 18, 19 (long-day plants) with Fig. 20 will show this approaching similarity to long-day metabolism in extended darkness. When placed in such conditions, the late-flowering chrysanthemum loses its carbohydrates in about the same time as do such long-day plants as calceolaria and wallflower.

D. The rates of starch-formation in long-day and short-day plants

Some experiments kindly performed with the writer by Miss Enid Clegg, B.A., suggest that the late-flowering chrysanthemum can form starch quicker than some early-flowering species. Plants were kept in the dark for 35 hr. until all starch had disappeared from the leaves, and were then exposed to sunlight. At hourly intervals one plant was removed to the darkness, and at subsequent half-hourly intervals samples of its leaves were removed and tested for starch by Sach's test. The result of such an experiment, carried out on 3 October 1933, is given in Table V.

Table V

Late-flowering chrysanthemum plants deprived of starch, and then placed in sunlight, 9.15 *a.m.*, 3 *October* 1933

Brought from sunlight to darkness	a.m. 10.15	10.45	11.15	11.45	p.m. 12.15	12.45	1.15	1.45	2.15	2.45	3.15	3.45	4.15	4.45
A. At 10.15 a.m.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B. ,, 11.15 a.m.	.	.	+	±	−	−			−		−			−
C. ,, 12.15 p.m.	.	.	.	.	+	±			−	−				−
D. ,, 1.15 p.m.	.	.	.	.	.	.	+	+	+	+	+	+	+	+
E. ,, 2.15 p.m.	.	.	.	.	.	.	.	.	+	+	+	+	+	+
Control (not exposed to sunlight)	−	−	−	−	−	−	−	−	−	−	−	−	−	−

It will be seen that starch appeared after 2 hr. exposure to sunlight, but disappeared almost immediately after it was placed in the dark. After 4 hr. in the light, it took more than $3\frac{1}{2}$ hr. to disappear after its removal to darkness.

More definite results were obtained on 4 and 5 October:

Date	Length of time for starch formation hr.	Length of time for starch removal hr.
4 Oct	2	More than $3\frac{1}{2}$
5 Oct.	1	Between 2 and 3

It is noteworthy that it takes roughly twice as long to remove the starch as it does to make it.

Experiments on *Primula wanda*, an early-flowering plant, and *Sedum spectabile*, a long-day species, carried out on 19 April 1936 in the writer's garden, seem to show that these plants were slower to make starch, and quicker to lose it:

Plant	Length of time for starch formation hr.	Length of time for starch removal hr.
Primula wanda	More than 4	2
Sedum spectabile	„ 4	2

It may be noted here that it takes about half the length of time to remove the starch as it does to make it.

Table VI portrays the climatic features of the days upon which the above experiments were carried out, and the writer thanks Miss Ellen Gallwey, of the Ravensknowle Museum, Huddersfield, for the figures.

Table VI

Meteorological data for experiments on the rate of starch formation

Figures for temperature and humidity are corrected to the nearest whole number. Data for October 1933 refer to experiments carried out at the Tolson Memorial Museum, Ravensknowle, Huddersfield, where the meteorological station is situated. Data for April 1936 apply to experiments carried out at the writer's garden, approximately ¼ mile north of the meteorological station.

	Temperature ° F.			Humidity %			Bright sunshine, hr.		Wind, m.p.h.		
Date	9a.m.	3p.m.	9p.m.	9a.m.	3p.m.	9p.m.	Sunrise–noon	Noon–sunset	9a.m.	3p.m.	9p.m.
1933											
3 Oct.	48	55	42	86	69	98	—	0·4	—	—	—
4 Oct.	52	61	56	82	81	90	0·5	0·7	—	9·6	—
5 Oct.	56	58	54	85	76	93	—	—	—	—	—
1936											
19 April	42	45	40	51	52	75	6·6	4·8	14	12·8	5

These results seem to warrant the conclusion that the late-flowering chrysanthemum has, in addition to delayed translocation, the somewhat ironical incubus of facile starch production. We can now visualize this plant as having such a rapid photosynthesis that the leaves quickly become gorged with starch, which is not removed to any great extent during the night. *Primula wanda* and *Sedum spectabile* are relatively slow manufactories of starch, but it is steadily removed. The fable of the hare and the tortoise is most apt.

SUMMARY AND CONCLUSIONS

1. The late-flowering chrysanthemum, a representative of the "short-day" group of plants, showed evidence of delayed nocturnal translocation, when compared with a number of summer-blooming species. In the late-flowering chrysanthemum transportable carbohydrate did not appear

during the short nights of summer, and was only detectable shortly before dawn in the long nights of spring. Starch was always present in the leaves. Transportation in early-flowering plants could be inferred either from nocturnal disappearance of starch, or the abundance of reducing sugars, or a periodicity of non-reducing sugars. Quantitative determinations of various carbohydrate fractions of early-flowering and late-flowering chrysanthemum confirmed the delayed translocation in the latter, using the surer criterion of nightly decrease in the total and insoluble carbohydrates from mature leaves.

2. Artificial lengthening of the night during summer causes late-flowering plants to bloom earlier, and it is suggested that this effect results from the increased facility for translocation afforded by the longer period in darkness.

3. The results explain why a period of 4 hr. darkness around midday is not effective in hastening the flowering of a short-day plant (Garner & Allard, 1931). Such a period is not long enough for effective translocation to begin, and the plant has approximately as much carbohydrate at the end of the period as at the beginning.

4. Preliminary experiments indicate that the late-flowering chrysanthemum forms starch much more easily than two long-day plants which have been tested. The short-day species has a surfeit of starch and poor translocation; the long-day plants have slower manufacture of starch, which, however, is steadily removed. These results provide a necessary link between the hypothesis that a high carbohydrate-nitrogen ratio tends towards flowering, and the facts of photoperiodism. It is possible that delayed translocation of the late-flowering chrysanthemum postpones the attainment of a suitable ratio until the long nights of autumn, or artificially lengthened nights in summer, increase the nightly period of effective carbohydrate translocation, and thus the necessary balance is attained. A suitable carbohydrate-nitrogen ratio can readily be attained by long-day plants, by virtue of their effective translocation.

5. The results also focus attention upon the necessity of estimating the *mobility* of carbohydrate in considering its effect upon time of flowering. It is not sufficient to estimate the soluble carbohydrate, nor even the total carbohydrate, for neither of these figures, taken by itself, would give any adequate idea of the availability of the carbohydrate for altering the ratio with nitrogen.

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OBSERVATIONS OF THE EFFECT OF NITROGEN AND POTASSIUM ON THE FRUITING OF THE TOMATO

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(With 12 Text-figures)

CONTENTS

Introduction

Previous literature on the influence of nitrogen and potassium on the fruiting of the tomato includes communications from Bewley & White (1926), who described the symptoms of nitrogen and potassium deficiency, and Owen (1929, 1931), who investigated the chemical composition of the fruit and foliage. In America the effect of variation in nitrogen supply has been considered by Kraus & Kraybill (1918), Murneek (1925, 1926), Nightingale (1927), Nightingale *et al.* (1928) and Clark (1936) and that of potassium supply by Johnston & Hoagland (1929), Janssen & Bartholomew (1929), Nightingale *et al.* (1930) and Phillips *et al.* (1934). With the exception of the work of Murneek, to which special reference

is made below, the conclusions of these investigators that are germane to the present observations are referred to as occasion arises in the presentation of the results.

Murneek (1926) concludes that there is a negative correlation between vegetative activity and fruiting. A plant carrying a heavy crop receives a check to vegetative development which continues until the crop has matured, a recurrence of this process leading to "cyclic growth" (1925). Murneek (1926) claims that the presence of one fruit only on a nitrogen-starved plant is sufficient to check further vegetative extension, and attributes this effect to the monopolization by the fruit of practically all the nitrogen absorbed and elaborated, combined with the inability of the tomato plant to store appreciable amounts of nitrogen. "It is evident, therefore, that *a condition of nitrogen starvation with all its attendant manifestations can be brought about in vegetative parts of the tomato by the correlative effects of the fruit, and quite independently of the external supply of nitrogenous nutrients*" (Murneek, 1926, p. 26). Murneek supports his views with some striking analyses. He shows that with abundant nitrogen supply 46 % of the total nitrogen of the plant is contained in the fruits,[1] so that in defruited plants the nitrogen content is raised from 29 to 57 % in the leaves, 14 to 30 % in the stems, and 7 to 13 % in the roots. Whereas the main factor involved in the checking of vegetative development in Murneek's experiments appears to have been the nitrogen supply, large amounts of other nutrients are contained in the fruits,[2] and it seems possible that under different conditions shortage of other nutrients, e.g. carbohydrates, might lead to similar checking of vegetative development. This possibility, suggested by Murneek (1926), has received experimental support from observations on the effect of variation in carbon dioxide supply (White, 1930).

Experimental procedure

In order to study the effect of manurial deficiency on fruiting the method of detailed observation of the behaviour of individual blossoms, suggested by Bewley & Corbett (1930), has been developed. Observations were made throughout the season of 1933 on the fruiting of plants grown on three plots, 12 ft. sq., separated by walls of concrete. One of these plots was fertilized with complete artificials; on the other two plots nitrogen and potassium respectively had been omitted from the scheme

[1] Owen (1929) finds 39 % for the English glasshouse tomato.

[2] Murneek gives the percentage soluble carbohydrates in the fruits at ten times that in the stems. Owen (1931) finds that 41 % of the total K_2O in tomato plants is in the fruits.

of manuring over a period of years.[1] Each plot contained fifty-six plants. In order to reduce the error of the observations the marginal rows were rejected, as also were the rows adjacent to two hot-water pipes running through the plots for heating purposes. Twenty-four plants remained for observation on the no nitrogen and no potassium plots. Owing to a slightly different arrangement of the paths on the completely manured plot six more plants could be included, making thirty for this treatment. The plants were trained up trellis suspended from the roof and their growth confined to the main axis by continuous removal of incipient axillary shoots. Under these circumstances the rate of extension of the main axis, estimated from periodical measurements of height, is a useful measure of growth rate. It is essential to emphasize the distinction between rate of "growth" in height, and rate of "development" of leaves and flower-trusses from the growing point, estimated from the times at which corresponding flower-trusses unfold. It was decided to continue observations to a developmental stage corresponding to the tenth truss (blossom cluster), since the height of the plants at later stages would have necessitated the use of a ladder, which was impracticable, while fruit on higher trusses was unlikely to mature before the onset of winter. Observations were commenced on seventy-eight plants and continued as far as possible daily throughout the season with the exception of Sundays, observations for these days being estimated from the previous and subsequent days. Records of fifteen plants had to be discontinued from time to time owing to destruction by accident and insect or fungal attack. Seeds were sown of the variety Ailsa Craig on 28 December. The seedlings were given normal manurial treatment in the seed boxes and in 3 in. pots to which they were transplanted on 19 January. Planting out into the different plots took place on 3 March. The first observation was made on 24 March and the final observation on 13 October following.

Observations of the effect of nitrogen and potassium

(*a*) *Number of blossom-buds and fruits*

Table I (columns 2, 3 and 4) and Fig. 1 give the mean number of blossom-buds formed per truss. The third trusses have the greatest number of blossom-buds and their formation is affected by the nitrogen supply, since the mean number throughout the season is consistently low for the nitrogen-starved plants. Blossom-bud formation is not

[1] For details of the manuring see the *Cheshunt Experimental Station Annual Reports*, 1916–29.

affected by low potassium supply, and this suggests that the growing points of potassium-starved plants receive an ample nitrogen supply throughout the season.

Table I (columns 5, 6 and 7) and Fig. 2 give the mean number of fruits formed per truss. The number of fruits is greatest for the second truss of the fully manured plants and falls as the season proceeds but shows partial recovery on the eighth and ninth trusses. Variation in number of fruits of the nitrogen-starved and potassium-starved plants follows a similar course, but the mean number is reduced by both nitrogen and potassium starvation.

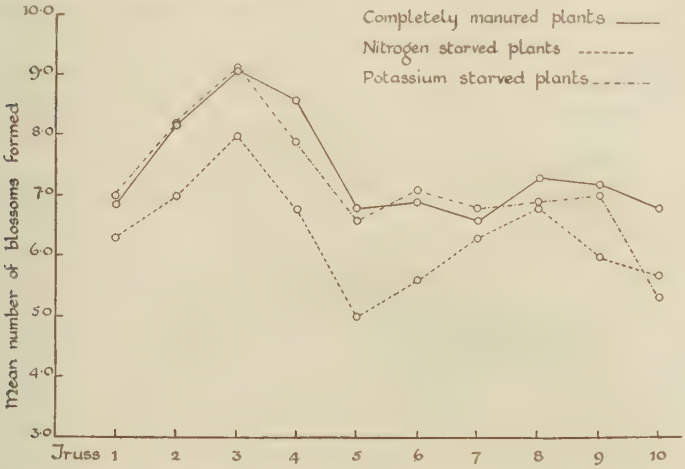

Fig. 1. Mean number of blossom-buds formed on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table I

C.A. = completely-manured plants
– N = nitrogen-starved plants
– K = potassium-starved plants

	Mean number of blossom-buds formed			Mean number of fruits per truss			% blossoms opening of buds formed		
Truss	C.A.	– N	– K	C.A.	– N	– K	C.A.	– N	– K
1	6·9	6·3	7·0	6·7	6·3	6·5	100	100	100
2	8·2	7·0	8·2	7·1	5·8	4·8	100	99	98
3	9·1	8·0	9·2	6·8	5·3	5·6	95	91	95
4	8·6	6·8	7·9	5·6	2·4	4·1	77	57	75
5	6·8	5·0	6·6	3·4	1·4	1·3	56	36	29
6	6·9	5·6	7·1	2·4	1·0	1·2	45	45	66
7	6·6	6·3	6·8	2·3	1·7	1·2	59	68	84
8	7·3	6·8	6·9	3·3	2·1	1·6	92	77	71
9	7·2	6·0	7·0	3·5	1·1	1·3	89	83	79
10	6·8	5·7	5·3	2·4	0·7	0·3	79	68	62

Fig. 2. Mean number of fruits borne on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

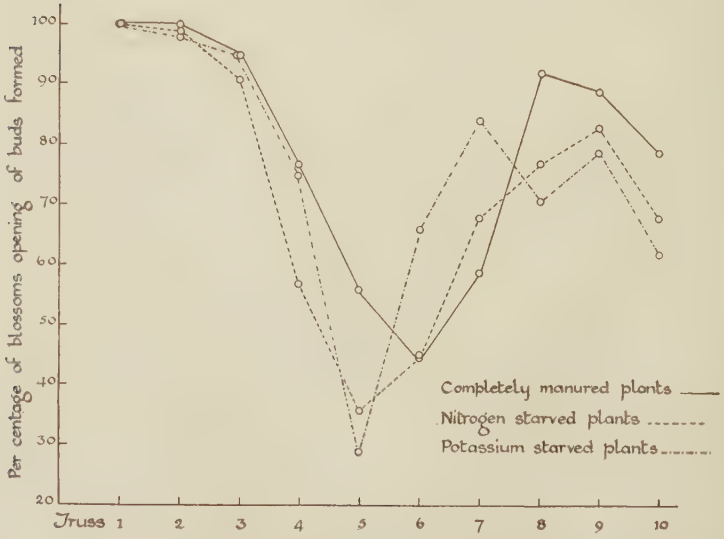

Fig. 3. Percentage blossoms opening of buds formed on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table I (columns 8, 9 and 10) and Fig. 3 give the percentage of blossoms opening of buds formed. Fig. 3 shows that for all three treatments this percentage falls sharply, reaching a minimum at the sixth truss of 45 % for the fully manured plants, and minima of 29 and 36 % at the fifth truss for the potassium-starved and nitrogen-starved plants. The high proportion of aborted buds, 55 % in the case of the fully manured plants, doubtless reflects competition for nutrient supply with the developing fruits of the second, third and fourth trusses. After maturation of these fruits a greater percentage of the buds formed on higher trusses are able to develop to the blossoming stage. On only one of the first seven trusses is the percentage of blossoms opening on potassium-starved plants markedly inferior to that of the fully-manured plants, and it appears, therefore, that the relatively low number of fruits on the potassium-starved plants (Fig. 2) is due to failure of pollination.

(*b*) *Rate of development of flower-trusses*

Records of dates of opening of the blossoms for each treatment throughout the season are too voluminous to reproduce in full: the means are given in Table II and Fig. 4. The number of blossoms opening per truss naturally varies with the individual plant, and the mean values given for apical blossoms are those where four or more replicates are available. The most satisfactory method of analysing these results would be to apply Fisher's analysis of variance (Fisher, 1935). This, however, has not been attempted as, even if only the first six blossoms of the trusses were included, the number of observations would approximate to 3 treatments $\times$ 10 trusses $\times$ 6 blossoms $\times$ 24 replicates = 4320. The significance of the differences between trusses may, however, be estimated by comparison of the differences between corresponding blossoms of different treatments by "Student's" method of t (Fisher, 1936). The results can then be combined for each truss by taking advantage of the fact that the variance of the sum of a number of means is equal to the sum of the separate variances.

The dates of opening of the blossoms in the fully manured plants may first be considered. Since, with the partial exception of the two intervals separating the basal three trusses (which correspond with a development of five leaves, in 32 and 21 % of plants respectively), three leaves separate each truss, the relative differences in time between the opening of the basal blossoms of the trusses afford a measure of the rate of development of the growing point throughout the season (see Appendix). These differences shorten until the fourth truss is reached, lengthen between

Table II

Mean dates of opening of blossom-buds throughout the season

Columns = fruits numbered from base to apex of the trusses
Rows = trusses numbered from base to apex of the plants

	1	2	3	4	5	6	7	8	9	10
					Completely manured plants					
1	29·3	8·4	17·4	25·4	5·5	14·5	28·5	13·6	19·6	27·6
2	31·3	10·4	18·4	26·4	6·5	16·5	2·6	14·6	21·6	28·6
3	2·4	11·4	19·4	27·4	7·5	17·5	9·6	15·6	23·6	29·6
4	4·4	12·4	20·4	29·4	9·5	20·5	13·6	16·6	24·6	1·7
5	5·4	14·4	22·4	1·5	10·5	21·5	13·6	18·6	25·6	2·7
6	7·4	15·4	23·4	2·5	12·5	25·5	16·6	20·6	26·6	4·7
7	9·4	16·4	25·4	5·5	—	13·6	18·6	24·6	27·6	3·7
8	—	18·4	27·4	6·5	—	—	—	29·6	29·6	—
9	—	20·4	28·4	8·5	—	—	—	—	—	—
					Nitrogen-starved plants					
1	29·3	9·4	19·4	28·4	9·5	12·6	5·7	17·7	1·8	13·8
2	31·3	10·4	20·4	28·4	10·5	15·6	5·7	20·7	2·8	12·8
3	2·4	12·4	21·4	29·4	11·5	22·6	8·7	23·7	4·8	14·8
4	4·4	13·4	22·4	30·4	13·5	30·6	12·7	25·7	7·8	16·8
5	5·4	15·4	24·4	3·5	—	3·7	16·7	27·7	8·8	18·8
6	7·4	16·4	25·4	3·5	—	5·7	17·7	28·7	12·8	18·8
7	8·4	18·4	26·4	—	—	—	18·7	28·7	11·8	—
8	—	18·4	27·4	—	—	—	—	—	—	—
					Potassium-starved plants					
1	28·3	6·4	14·4	22·4	3·5	14·5	24·5	2·6	9·6	20·6
2	29·3	8·4	15·4	23·4	3·5	15·5	26·5	3·6	11·6	21·6
3	31·3	9·4	16·4	24·4	7·5	18·5	28·5	4·6	12·6	21·6
4	2·4	10·4	18·4	25·4	10·5	20·5	30·5	5·6	13·6	22·6
5	4·4	12·4	19·4	27·4	17·5	23·5	1·6	7·6	16·6	—
6	6·4	14·4	21·4	29·4	—	26·5	2·6	8·6	18·6	26·6
7	8·4	15·4	22·4	2·5	—	28·5	6·6	10·6	16·6	—
8	—	17·4	24·4	—	—	—	—	—	—	—
9	—	20·4	24·4	—	—	—	—	—	—	—

the fourth and seventh trusses and then shorten again until the ninth truss. Corresponding differences may be seen in the behaviour of later blossoms of each truss. The periods between the opening of adjacent blossoms in truss 1 approximate to regular 2-day intervals, and it is apparent that the slopes of the curves afford a measure of the rate of development of each truss. The steepest slope is recorded for truss 2, and, thereafter, the slopes show a progressive falling off until there are signs in truss 6 of a marked retardation of development. This process is accentuated in the early blossoms of truss 7. The mean slope of truss 8 is higher, and the intervals of truss 9 do not differ from those of truss 3 before the retardation sets in. The period of greatest retardation lies between 21 May and 13 June, corresponding to and slightly preceding (since the basal blossom of the youngest truss opens when situated a few cm. from the growing point) the opening of blossoms on the seventh and eighth trusses. This period synchronizes with the final stages of develop-

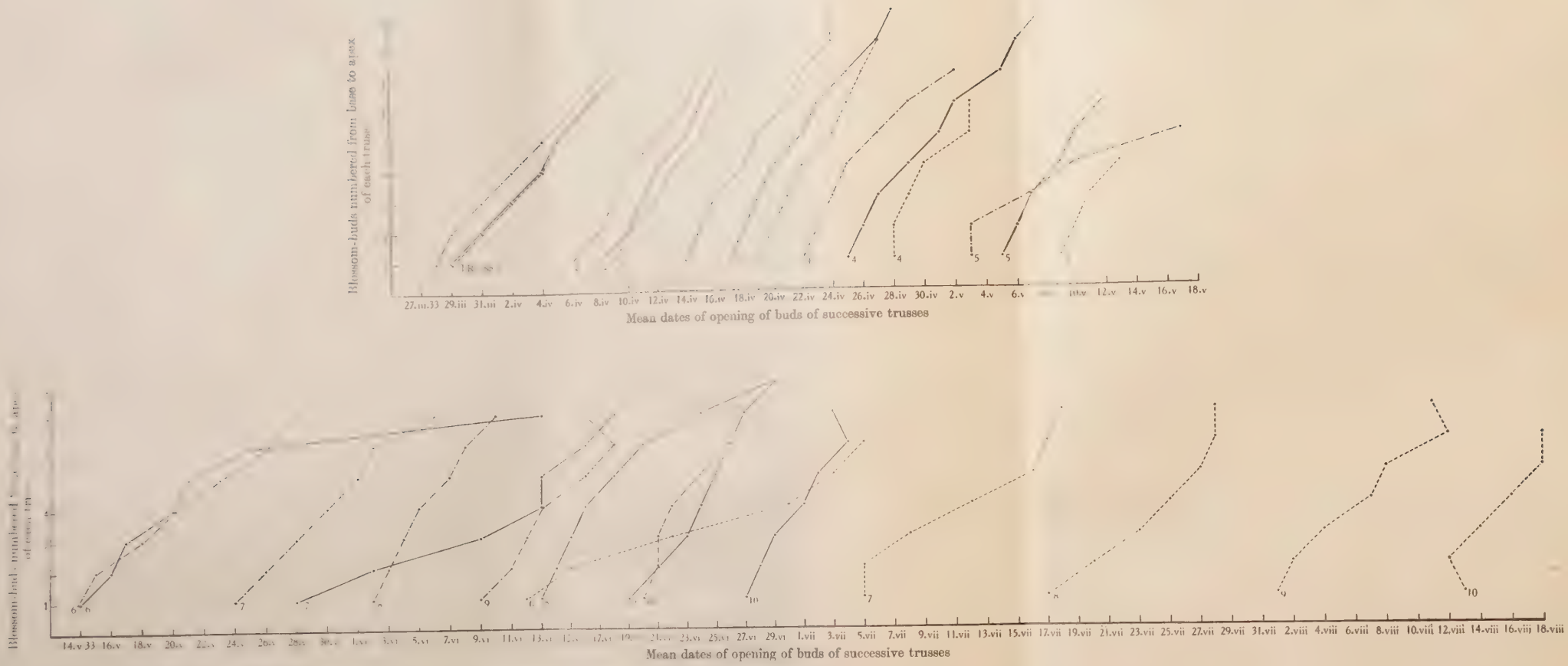

Fig. 4. Dates of opening throughout the season of successive blossoms of successive trusses of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants. All values are the means of four or more replicates.
Completely-manured plants ——; nitrogen-starved plants - - -; potassium-starved plants —·—.

ment of the fruit on trusses 2, 3 and 4, which together constitute the major portion of the crop.

Comparison of the rates of development of completely manured plants with potassium-starved and nitrogen-starved plants in Fig. 4 reveals several striking features.

(1) The blossoms of potassium-starved plants open *earlier* than corresponding blossoms of completely manured plants. Differences of as much as 11 days between corresponding trusses are recorded in favour of the potassium-starved plants. In order to remove any doubt as to the significance of this effect, "Student's" method of t (Fisher, 1936) has been used to compare the means of corresponding blossoms of truss 3, which show differences of about 3 days. The results are shown in Table III (columns 2, 3, 4 and 5), the low values of P demonstrating the precision that may be obtained by the use of a large number of replicates. There is clear evidence of an acceleration by potassium starvation of the rate of development of successive flower trusses from the growing point.

Table III

Comparison of differences in dates of opening of blossom-buds (numbered from base to apex) of third truss by "Student's" method of "t"

	Completely manured and potassium-starved plants				Completely manured and nitrogen-starved plants			
Fruit no.	Mean difference in days	n_1+n_2	t	P	Mean difference in days	n_1+n_2	t	P
1	3·0	43	3·51	<0·01	2·0	46	2·32	0·02
2	3·0	43	3·51	<0·01	2·0	46	2·24	0·03
3	3·0	43	3·39	<0·01	2·0	46	2·20	0·03
4	2·0	43	2·36	0·03	2·0	45	2·10	0·04
5	3·0	43	3·03	<0·01	2·0	45	1·90	0·06
6	2·0	43	1·97	0·05	2·0	44	1·92	0·06
7	3·0	43	2·89	<0·01	1·0	41	0·87	—
8	3·0	40	2·80	<0·01	±0·0	35	—	—
9	4·0	26	5·28	<0·01	—	—	—	—

(2) The check to rate of development in mid-season and subsequent recovery is *accelerated* in potassium-starved plants. The trusses showing the greatest retardation are the fourth and fifth as against the sixth and seventh of the completely manured plants. Recovery is nearly complete in the seventh truss of the potassium-starved plants which takes 13 days for the opening of seven blossoms, whereas the opening of seven blossoms of the completely manured plants takes 21 days. The intervals between the opening of blossoms of the eighth truss of the potassium-starved plants do not differ from those of truss 3 before retardation sets in,

whereas the intervals between the opening of blossoms of truss 8 of the completely manured plants reveal definite signs of retarded development.

(3) The blossoms of nitrogen-starved plants, with the exception of those of the first truss, open *later* than corresponding blossoms of completely manured plants. In order to test the significance of this effect, in the early part of the season before the checking of development sets in, the means for the blossoms of truss 3 are compared in Table III (columns 6, 7, 8 and 9) with the corresponding means for the blossoms of the completely manured plants. The value of t for the whole truss, obtained from a comparison of the sums of the means, is 4·31, which corresponds with $P = < 0{\cdot}01$. Nitrogen starvation and potassium starvation thus have contrasting effects on the rate of development of successive trusses from the growing point.

(4) A remarkable feature of Fig. 4 is the influence of nitrogen on the mid-seasonal checking of vegetative development, which is common to all treatments and has been associated with competition for nutrient supply with the developing fruits of trusses 2, 3 and 4. The first effect of nitrogen starvation is failure of the buds formed to open, so that the fifth truss of the typical nitrogen-starved plant consists of only four blossoms. At this stage development practically ceases, for the basal blossom of the next truss does not open until more than 4 weeks later. Thus the rate of development of the growing point must be so slow as to correspond with the unfolding of only three leaves in 4 weeks. Moreover, the sixth truss takes 23 days to open. Succeeding trusses recover slowly, but their slopes do not reach the levels of those trusses that blossom prior to the mid-seasonal check. The differences between the treatments become so pronounced that the basal blossom of the tenth truss of a typical nitrogen-starved plant opens about 2 months later than the corresponding blossom of a typical potassium-starved plant.

The marked retardation of blossoming associated with nitrogen starvation demonstrates that nitrogen is a factor of prime importance in determining the rate of development of flower-trusses from the growing point. The accentuation of the mid-seasonal check by nitrogen starvation is in agreement with the conclusions of Murneek (1926) that developing tomato fruits tend to monopolize the nitrogen supply bringing about a condition of nitrogen starvation in other parts of the plant.

(c) Relative effect of starvation throughout the season

The favourable development of the potassium-starved plants in comparison with the completely manured plants raises the question of to what extent were the plants on the no potassium plot really potassium-starved. Observations were made on fruit developing from the blossoms noted in Table II and Fig. 4. When mature this fruit was graded by eye into four classes corresponding to those used in the commercial marketing of tomatoes. From the mean weight of these grades the total weight of fruit corresponding with each truss for each treatment has been calculated. The weight of crop of the nitrogen-starved and potassium-starved plants, estimated as a percentage of the fruit borne by the completely manured plants, is shown in Fig. 5, which possesses several points of interest.

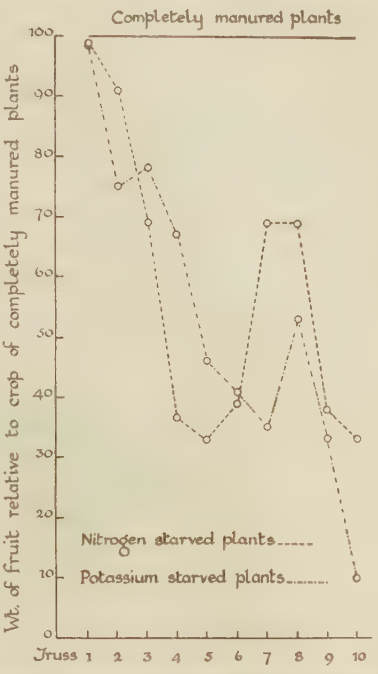

Fig. 5. Fluctuation in weight of fruit, relative to that of corresponding trusses of completely manured plants, of different trusses (numbered from base to apex of the plants) of (*a*) nitrogen-starved plants, and (*b*) potassium-starved plants.

(1) The plants on the potassium-starved plots are suffering so severely from potassium starvation that the crop on the seventh, eighth, ninth and tenth trusses is only 30 % of that of the completely manured plants. It is clear that the conditions controlling development of floral organs are very different from those subsequently necessary for the production of a heavy crop.

(2) In so far as weight of crop may be used as an indication of the degree of starvation there is no essential difference in degree of starvation between the nitrogen-starved and potassium-starved plants. The starvation effect is negligible for the first truss but increases rapidly as the season advances.

(3) There is clear indication in the case of the nitrogen-starved plants of an accentuation of the starvation effect on the fourth, fifth and sixth

trusses and a recovery on the seventh and eighth trusses. The relative effect of a low nitrogen supply is thus most marked at the period corresponding to heavy bearing of fruit. It is evident that the form of the curve showing the percentage relationship of the crop of the nitrogen-starved plants to that of the completely manured plants is that expected on the view that development of a heavy crop is controlled by the available nitrogen supply.

(*d*) *Rate of growth*

Table IV and Fig. 6 give the mean heights of the plants on each plot from 10 April until further measurements became impracticable owing to the height of the completely manured plants. Growth measurements corresponding to all treatments clearly represent two stages—a period of progressive falling off terminating in almost complete cessation of growth, followed by a period of recovery, when the relationship between height and time becomes linear. It is of interest to compare these results with the corresponding weights, shown in Table V, of mature fruit picked. The maximal weights of fruit matured for any 6 days during the season were picked between 27 May and 2 June, and this period approximates closely to that of recovery in growth rate.

Table IV

Mean height of plants each trimmed to a single axis (cm.)

Date	Completely manured	Nitrogen-starved	Potassium-starved
10 April	68	55	69
17 „	89	73	87
24 „	104	83	97
1 May	120	93	103
8 „	128	95	107
15 „	138	97	115
22 „	141	98	122
29 „	144	98	132
5 June	156	101	141
12 „	173	104	147
19 „	179	106	157

Table V

Weight of fruit in oz. maturing during successive 6-day periods

Date	Completely manured	Nitrogen-starved	Potassium-starved
6–12 May	—	8	10
13–19 „	72	111	85
20–26 „	358	384	316
27 May–2 June	478	349	353
3– 9 June	334	152	118
10–16 „	271	102	125
17–23 „	65	23	76

At a period corresponding to development of the second truss there is no difference in rate of growth between the completely manured and potassium-starved plants. Subsequently, the falling off in rate of growth of the potassium-starved plants is more severe and the slope of the curve after recovery is less steep. *Potassium starvation is thus responsible at the same time for acceleration of development* (Fig. 4) *and retardation of growth* (Fig. 6). The retardation of growth of the nitrogen-starved plants during the latter part of May is striking and emphasizes the importance of the effect of nitrogen supply on the severity of vegetative checking, that is associated with heavy bearing of fruit.

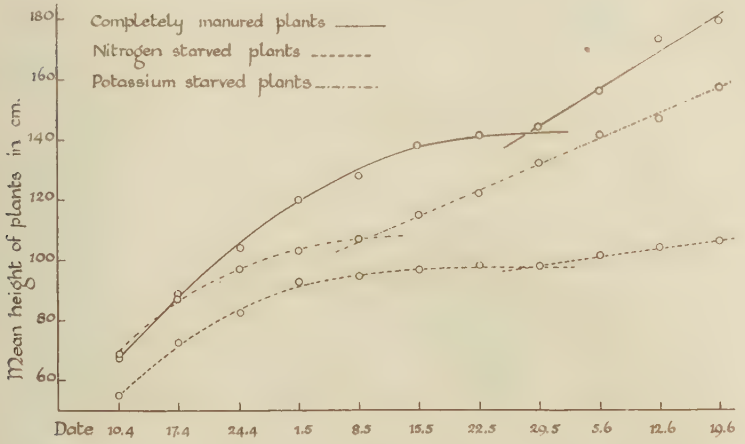

Fig. 6. Mean height in cm., at weekly intervals from 10 April to 19 June, of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

(*e*) "*Maturation*" *period of fruits*

Observations on the process of fruiting were continued by noting the dates on which each fruit became mature. Maturity was taken as corresponding to the first appearance of the orange-red pigment of the fully ripe tomato, commercial fruit being usually picked at this stage. The period between the opening of the blossom and the picking of the fruit has been termed by Bewley & Corbett (1930) the "maturation period".

As pointed out by Bewley & Corbett (1930) and White (1930) the maturation periods of fruits developing on the second, third and fourth trusses do not show regular intervals from the basal to apical fruits of a truss. The maturation periods of the basal fruits are relatively constant

and there is then a marked break of from 15 to 25 days before the apical fruits mature. White (1930) suggested that this break was due to a shortage of nutrient material, the development of the later formed fruits being held up until the basal fruits had matured.

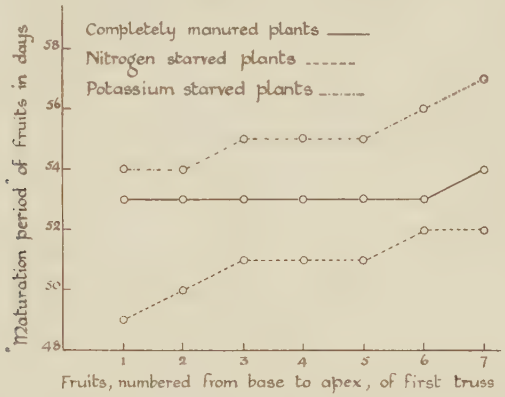

Fig. 7. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the basal truss of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table VI

Maturation period of fruits of completely-manured plants (C.A.), *nitrogen-starved plants* (−N) *and potassium-starved plants* (−K)

	Truss 1			Truss 2 (retarded apical fruit in brackets)			Truss 3		
Fruit	C.A.	−N	−K	C.A.	−N	−K	C.A.	−N	−K
1	53	49	54	50	50	54	52	53	57
2	53	50	54	51	50	54	52	54	57
3	53	51	55	52	51	56	51	55	—
4	53	51	55	53	51 (82)	57 (81)	52	—	61
5	53	51	55	54	54 (90)	— (80)	52	—	—
6	53	52	56	55 (80)	— (84)	—	53	—	—
7	54	52	57	55 (87)	— (85)	—	—	—	—
8	—	—	—	— (93)	–	—	–		—

Table VI (columns 2, 3 and 4) and Fig. 7 show the maturation period of fruits of the first truss. For the completely manured plants the maturation period is constant at 53 days. The mean maturation period of the basal fruits of the nitrogen-starved plants corresponds with a gain of 4 days in ripening, but this difference tends to be lost as the later-formed fruits mature. The mean maturation period of the basal fruit of the potassium-starved plants is 1 day longer than that of the completely

manured plants, and this difference is accentuated with later-formed fruit. "Student's" method of t (Fisher, 1936) has been used to test these differences and the results are shown in Table VII. The maturation period of the nitrogen-starved plants is significantly low for six of the seven blossoms. The maturation period of the potassium-starved plants, excluding the first two fruits, is significantly high.

Table VII

Comparison of differences in period of ripening of fruits (numbered from base to apex) of first truss by "Student's" method of "t"

	Completely manured and nitrogen-starved plants				Completely manured and potassium-starved plants			
Fruit no.	Mean difference in days	n_1+n_2	t	P	Mean difference in days	n_1+n_2	t	P
1	4·0	44	4·96	<0·01	1·0	41	1·47	0·14
2	3·0	43	5·19	<0·01	1·0	39	1·51	0·14
3	2·0	43	3·16	<0·01	2·0	40	2·70	0·01
4	2·0	44	3·70	<0·01	2·0	41	2·58	0·02
5	2·0	43	3·44	<0·01	2·0	39	3·46	<0·01
6	1·0	42	1·54	0·12	3·0	39	3·69	<0·01
7	2·0	25	2·76	0·01	3·0	27	3·42	<0·01

Table VIII

Mean maturation period for different trusses (excluding retarded apical fruit)

Truss	Completely manured	Nitrogen starved	Potassium starved
1	53	51	55
2	52	51	55
3	52	51	59
4	52	51	58
5	57	56	57
6	56	53	56
7	53	55	55
8	52	54	59
9	51	54	58
10	51	56	59

Table VI (columns 5, 6 and 7) and Fig. 8 give the maturation period of the second truss. The values for the completely manured fruits contrast with those for truss 1, since they lengthen regularly as the later-formed fruits mature. The retardation in ripening of the potassium-starved plants is accentuated as compared with truss 1 and the low maturation period of the nitrogen-starved plants is tending to disappear. The means of the apical "retarded" fruit are plotted separately and do not fall below 80 days for any treatment.

The mean maturation periods for the (non-retarded) fruit of each truss are given in Table VIII and Fig. 9. Potassium starvation is

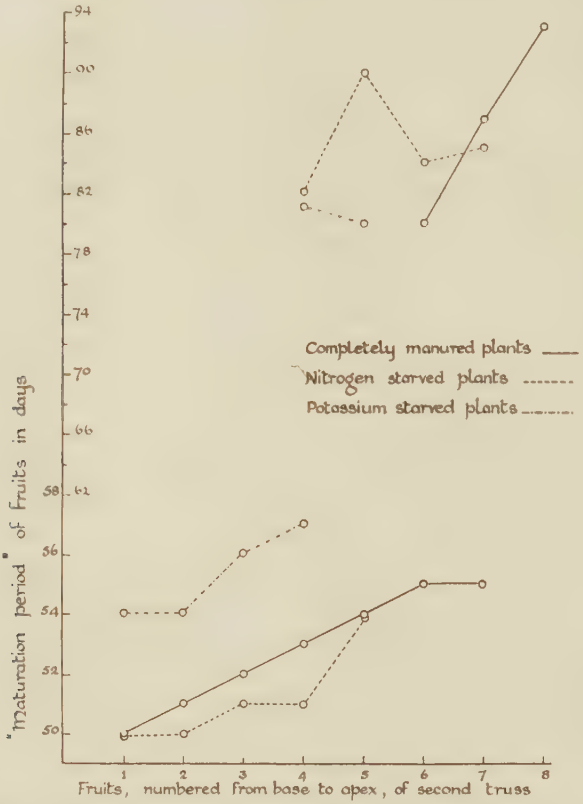

Fig. 8. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the second truss (from the base) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants. The striking difference in ripening period between the basal fruits and those apical fruits which show retarded development (plotted separately) may be noted. The values for these "retarded" apical fruits are plotted on half-scale in order to bring them on to the same diagram.

associated throughout the season with retardation in the ripening period and the acceleration in ripening of the fruit of the lower trusses of the nitrogen-starved plants is lost as the season advances. The ripening period of the completely manured plants is appreciably prolonged for

trusses 5 and 6 but subsequently shows complete recovery and is apparently independent of the age of the plant.

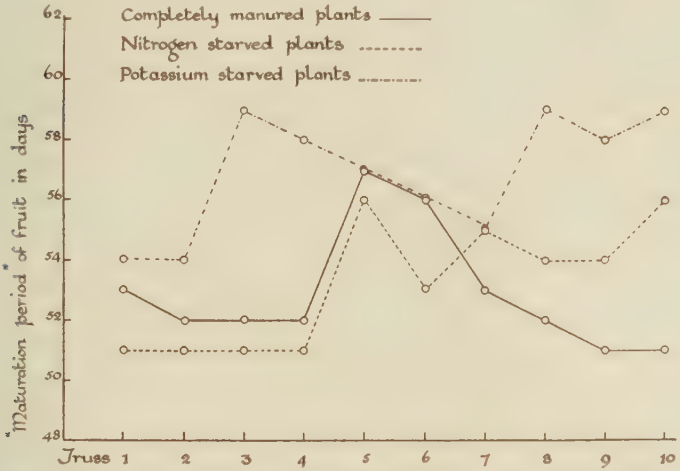

Fig. 9. Mean maturation period of fruits (excluding apical fruits with arrested development) of different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

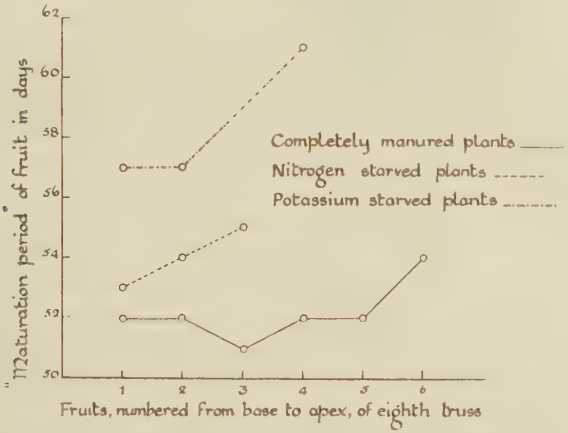

Fig. 10. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the eighth truss (from the base) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table VI (columns 8, 9 and 10) and Fig. 10 give the maturation period of the fruits of the eighth truss. The maturation period of different

fruits of the completely manured plants approximates to a constant as in truss 1, whereas all intermediate trusses show a tendency to lengthen for later-formed fruits, in addition to a well-marked prolongation in ripening of the apical "retarded" fruits.

Table IX and Fig. 11 give the relative number of apical fruits that were retarded in development until the basal fruits of their trusses had

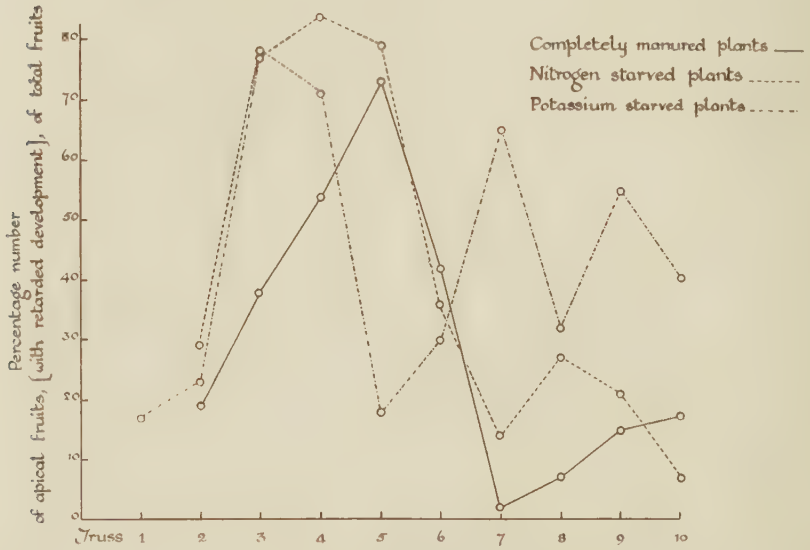

Fig. 11. Mean numbers of fruits with retarded development as percentages of the total numbers of fruits borne on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table IX

Numbers of fruits of different trusses: (a) ripening normally, (b) with arrested development

	Completely manured plants		Nitrogen-starved plants		Potassium-starved plants	
Truss	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
1	166	1	132	—	114	2
2	143	34	87	35	63	19
3	106	64	26	85	21	74
4	64	76	8	43	20	49
5	23	61	6	23	18	4
6	34	25	14	8	14	6
7	56	1	30	5	7	13
8	77	6	33	12	19	9
9	74	13	19	5	10	12
10	50	10	13	1	3	2

matured. The proportion of "retarded" apical fruit is very high for all treatments on the third, fourth and fifth trusses. These are the trusses that carry a heavy crop, and the percentage of "retarded" fruits falls when this crop has matured. A high proportion of these fruits occurs earlier in the nitrogen-starved plants in comparison with the completely manured plants, while the rise and subsequent fall is to a higher level than that of completely manured plants. It is of interest to note that the maximal percentage of "retarded" apical fruits is shifted towards the lower trusses in the potassium-starved plants. Fig. 11 shows that the proportion of "retarded" apical fruit is less than 20% for the fifth truss of the potassium-starved plants, whereas it is still over 70% for the corresponding truss of the completely manured plants. The production of "retarded" apical fruit is accentuated by both low nitrogen supply and low potassium supply.

(*f*) *Leaf area*

Since it was impracticable to use whole leaves the area of corresponding leaflets was estimated by enclosing leaves between thin panes

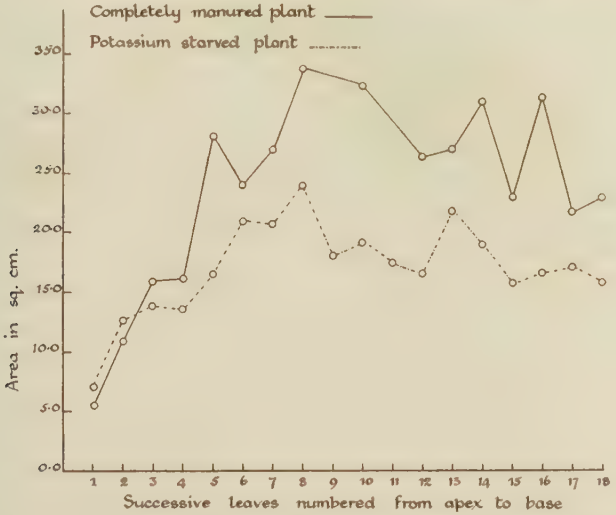

Fig. 12. Mean area in sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base, of (*a*) a completely manured plant, and (*b*) a potassium-starved plant.

of glass, drawing an illuminated outline on tracing paper and tracing the areas with a planimeter. Owing to pressure of other work and the large number of estimations that would have been required, the work had to

be limited to comparison of a single plant from each treatment. Care was taken to select a normal completely manured plant and plants with characteristic though moderate symptoms of nitrogen and potassium starvation. The terminal pair of leaflets of every leaf on the plants was used.

Table X

Area (sq. cm.) of corresponding leaflets from every leaf (numbered from apex to base) of a typical plant from each treatment

Leaf no.	Completely manured	Nitrogen-starved	Potassium-starved
1	5·67 5·58	6·25 5·83	5·90 6·96
	5·48	5·41	7·03
2	11·60 10·8	12·7 12·7	11·7 12·6
	9·99	12·8	13·5
3	15·7 15·8	15·4 15·4	14·6 13·8
	15·9	15·5	12·9
4	15·0 16·2	19·5 18·7	12·6 13·6
	17·5	17·9	14·6
5	27·0 28·0	18·6 18·8	16·0 16·6
	29·0	19·1	17·3
6	24·2 23·9	13·4 13·5	21·8 20·9
	23·6	13·7	19·9
7	26·4 26·9	20·1 18·8	21·6 20·7
	27·4	17·5	19·8
8	34·4 33·7	18·6 18·7	21·6 23·9
	33·0	18·9	26·2
9	31·5 32·3	24·1 24·1	19·1 18·1
	33·1	19·0	17·1
10	26·0 26·3	23·0 22·5	21·4 19·2
	26·6	22·0	16·9
11	26·6 27·0	16·1 16·1	18·3 17·4
	27·5	—	16·4
12	30·9 31·0	19·1 17·9	16·0 16·6
	31·2	16·6	17·2
13	23·4 23·0	15·5 15·8	21·8 21·8
	22·6	16·2	21·7
14	28·8 31·3	—	16·6 19·0
	33·8		21·0
15	21·1 21·7	—	17·9 15·7
	22·3		13·5
16	22·9 22·9	—	17·4 16·6
	23·0		15·7
17	—	—	16·2 17·1
			18·1
18	—	—	14·8 15·7
			16·6

The results are given in Table X, which shows that the leaflets corresponding to the eighth leaf from the growing point of the completely manured plant have the greatest area and are presumably the youngest to have reached their full size. Fig. 12 shows that the greatest difference in area between the fully manured and potassium-starved plants occurs at the eighth leaf from the growing point. The decline in area in older leaflets shows that up to the period of development of the fifth truss,

when these estimates were made, successive leaves attained a progressively larger size on reaching maturity. The area of *fully matured* leaves of the potassium-starved plant continues therefore to fall off relatively to the completely manured plant as the season advances. On the other hand, the *immature* leaves of the potassium-starved plant show with decreasing age progressively less difference in area relative to the completely manured plant.

DISCUSSION OF THE EFFECT OF NITROGEN AND POTASSIUM ON FRUITING

Effect of nitrogen supply

The observations of the preceding sections are summarized below:

(1) The rate of development of flower-trusses from the growing point and the rate of increase in height of plants trimmed to a single axis are both related to the nitrogen supply, becoming slower as the season advances and the effect of nitrogen starvation becomes more acute (Tables II, IV and Figs. 4, 6).

(2) The number of blossom-buds formed, the proportion of blossom-buds formed that open, and the number of buds formed that develop into fruit are related to the nitrogen supply, being in general low in nitrogen-starved plants (Table I and Figs. 1–3).

(3) The "maturation period" (between the opening of the blossom and ripening of the fruit) is shortened by slight nitrogen deficiency but lengthened by severe nitrogen starvation (Tables VI–VIII and Figs. 7, 9).

(4) The effect of low nitrogen supply on growth and on weight of crop is particularly marked at the mid-seasonal period of heavy fruit-bearing (Table V and Figs. 5, 6).

(5) The proportion of apical fruit that are severely retarded in ripening is increased by low nitrogen supply (Table IX and Fig. 11).

(6) Leaf area is markedly reduced by low nitrogen supply (Table XIV).

These observations lead to the following conclusions:

(1) Low nitrogen supply is associated with retardation of growth and development. Every characteristic of fruiting considered is retarded by low nitrogen supply, save the period of ripening of the fruit, and this is accelerated only in the lowest trusses. Increasing severity of nitrogen starvation later in the season is associated with prolongation of the ripening period.

The writer showed in a previous publication (White, 1930) that enrichment of the atmosphere of the glasshouse with carbon dioxide led

to a shortening of the period of ripening of the lowest trusses. Thus the period of ripening of the earliest formed trusses may be shortened *either by increasing the carbon dioxide supply in the atmosphere or by reducing the nitrogen supply in the soil.* Moreover, Porter (1937) has recently shown that a similar result is associated with increase of light intensity. This effect of a high carbohydrate-nitrogen balance accords with the view that the process of ripening is accelerated by a high level of sugars passing to the fruit. In a "vegetative" plant a higher proportion of the sugars synthesized in the leaves would tend to be used in vegetative growth and a lower proportion translocated to the fruit while the converse tendency would hold for a "fruitful" plant. The prolongation of the ripening period in the potassium-deficient plants thus suggests that, in these plants, there is impaired translocation of sugars to the fruits.

(2) The check to growth and development associated with heavy fruit-bearing on the second, third and fourth trusses is related to the nitrogen supply. In nitrogen-starved plants cessation of growth is more severe, reduction in crop of the fifth and sixth trusses is accentuated, and there is a high proportion of "retarded" apical fruits on the second, third and fourth trusses. These observations are compatible with the analyses by Owen (1929, 1931) of foliage of plants grown on these experimental plots in previous years, for leaves taken near the level of the fifth truss in June, i.e. at a period of heavy fruiting (Owen, 1929, Table I, 1924–5–6) have a lower nitrogen level than leaves taken in August, i.e. at a period of relatively light fruiting (Owen, 1929, Table I, 1927). Further the nitrogen level throughout the growing season falls to a minimum in the latter half of May with subsequent recovery (Owen, 1931, Table III). A previous publication (White, 1930) showed that a high level of carbon dioxide in the atmosphere of the glasshouse tended to reduce the severity of the mid-seasonal check, which seems therefore to be due to a general low level of nutrition. It appears that fruiting proceeds unregulated by the capacity of the plant to bear the number of blossoms opened and fruit set. The second, third and fourth trusses unfold at a period of the season when rates of growth and development are rapid and the intervals between the opening of blossoms are relatively short. This leads to the setting of a large number of fruits in a relatively short period. The demand for sugars and protein by the developing fruits increases until it exceeds the supply which is sufficient, even with temporary cessation of development of further trusses, for only a portion of the fruit set. A characteristic feature of fruiting in the

tomato now becomes apparent. The basal fruits of the trusses become possessed of the capacity to monopolize the nutrient supply, vegetative extension practically ceasing, differentiation of leaves and flower-trusses from the growing point being brought to a standstill and the apical fruits remaining in a state of suspended development. Further vegetative activity is apparent only when the crop is reaching maturity. A second period of relatively heavy fruit-bearing, associated with similar but less intense checking of vegetative growth and development, follows later in the season.

Regulation of fruiting is practised in the commercial culture of certain crops. Thus it is well known that in the apple the problem of biennial cropping, associated with a cycle of exhaustion and recovery, may be overcome by "thinning". There appears to be no reason why cyclic cropping of the tomato, due to alternating periods of exhaustion and recovery, should not be corrected by similar methods. It seems probable that, if the number of fruits on the lower trusses were restricted to those likely to reach good size and quality, an earlier and more even pick would be obtained and also, ultimately, a larger marketable crop, since the elimination of a large number of small fruits that do not reach marketable size would result in conservation of nutrient supply and less complete cessation of growth in mid-season.

Effect of potassium supply

The observations of the preceding sections are summarized below:

(1) The rate of development of flower-trusses from the growing point is related to the potassium supply, being consistently accelerated in potassium-starved plants (Table II and Fig. 4).

(2) The number of blossom-buds formed is not affected by the potassium supply, fluctuations in bud formation throughout the season in potassium-starved plants being almost identical with those of completely manured plants (Table I and Fig. 1).

(3) The percentage of blossoms formed that open is not appreciably affected by low potassium supply for the first seven flower-trusses, but the percentage of blossoms opening that fail to develop into fruits is consistently reduced in potassium-starved plants, apparently through failure of pollination (Table I and Figs. 2, 3).

(4) Fruit bearing is greatly affected by potassium supply, the weight of crop being markedly and progressively reduced throughout the season in potassium-starved plants (Fig. 5).

(5) The rate of growth of potassium-starved plants, estimated from increase in height, is not appreciably affected at a stage of development corresponding to the unfolding of the second flower-truss but subsequently shows a decline in relation to the completely manured plants (Table IV and Fig. 6).

(6) The "maturation period" of the fruit is prolonged in potassium-starved plants (Tables VI–VIII and Figs. 7–10).

(7) A high proportion of fruit in a stage of arrested development occurs earlier and on lower trusses in potassium-starved plants than in completely manured plants (Table IX and Fig. 11).

(8) The onset of and recovery from the check to vegetative growth, associated with heavy fruit-bearing on the second, third and fourth trusses, is accelerated by low potassium supply (Tables II, IV and Figs. 4, 6).

(9) Leaf area is related to the potassium supply. The maximal effect of low potassium supply is shown by the youngest mature leaf, older leaves, presumably associated with lesser degrees of severity of potassium starvation, being progressively less affected. The effect of low potassium supply on reduction in leaf area also becomes progressively less with decreasing age of leaf (Table X and Fig. 12).

An outstanding feature of these observations is the acceleration of differentiation of leaves and flower-trusses from the growing point by low potassium supply. Subsequent growth, estimated from the area of mature leaves and the length of the ripening period of the fruit, is retarded. Although development of the growing point of the main axis, estimated from the times of opening of blossom-buds of successive trusses is accelerated, growth, estimated from height, is retarded. These processes of acceleration of development and retardation of growth in height are clearly shown (Figs. 4, 6) to be taking place *simultaneously*.

The dependence of the rate of development of flower-trusses from the growing point upon the nitrogen level is shown by the striking retardation of development of the nitrogen-starved plants (Fig. 4). Even with moderate nitrogen starvation there is a significant retardation of development apparent as early in the season as the opening of the blossoms of the third flower-truss (Table III) although carbohydrate level must have been high at this period, as reflected in the shortening of the ripening period of the first flower-truss (Tables III, VI and VII). It would appear that a rate of development in advance of that of completely manured plants, as with potassium starvation, must be associated with high protein level at the growing point.

These results are attributed to the following effects:

(1) Potassium is continually translocated from the older portions of the plant to the growing point. This assumption may be made with some degree of confidence for it is generally accepted to be a characteristic feature of potassium starvation in plants. Nightingale *et al.* (1928) find by microchemical tests that nearly all the potassium in tomato plants starved of this element, apart from that contained in the fruit, is localized in the stem tip and in the younger leaves. Similar results are recorded by Janssen & Bartholomew (1929). The potassium in starved plants is apparently undergoing a continuous cycle, being translocated from older parts of the plant to the growing point, incorporated in young organs and subsequently retranslocated as these organs are approaching maturity.

(2) The level of potassium supply regulates nitrogen metabolism, a moderately low potassium supply being associated with high protein level and a greater degree of severity of potassium starvation with low protein level. By plunging leaves into hot water, decolorizing in warm alcohol and staining with Millon's reagent it was confirmed, from the intensity of the stain, that older potassium-starved leaves had a low protein level while the protein level of the young leaves was found to be at least as high as that of the young leaves of the fully manured plants. Similar conclusions have been reached by Richards & Templeman (1936), who have carried out detailed chemical analyses of potassium-starved barley leaves.

The combination of these two effects readily accounts for the results. Continual translocation of potassium from the older leaves to the young tissues maintains the growing point in the high protein phase. Consequently leaves and flower trusses of the potassium-starved plants are differentiated earlier than those of the completely manured plants, and the immature leaves show no reduction in area. In contrast mature leaves and older parts of the stems, from which the potassium is being translocated, have a lower protein level than the corresponding parts of completely manured plants. Growth in height involves the extension of the stem for some distance below the growing point, as indicated by the progressive lengthening of the internodes between successive leaves. Consequently, although the development of the growing point itself is accelerated in potassium-starved plants, the height of the plants is simultaneously reduced.

Reference may be made to the characteristic features of the foliage symptoms of potassium-starved plants in these experiments. In the early part of the season moderate potassium starvation is associated

with the production of leaves of a deep green colour presumably signifying high protein content, since the depth of colour and protein content of leaves vary together, as shown by Gassner & Goeze (1934), Michael (1935) and others. Owing to translocation to the growing point the concentration of potassium is subsequently reduced below that requisite for the maintenance of a high protein level, and this effect is first apparent as a marginal chlorosis indicative of protein breakdown.[1] As translocation of potassium proceeds chlorosis extends inwards, as described by Bewley & White (1926), associated with marginal drying out of the tissues or "scorch", until, finally, only the main veins retain their deep green colour. As the quantity of fruit hanging on the plant increases, the nitrogen level becomes insufficient to permit of both vegetative growth and fruit development and the concentration of protein at the growing point falls, associated with retardation of vegetative growth. At this stage very small leaves are produced of a pale yellow colour which resemble closely the leaves of plants on the nitrogen-starved plot. Later in the season, when the weight of fruit hanging on the plants is less heavy, larger leaves of a deep green colour are again produced.

These effects are in agreement with the results of other investigations of the influence of potassium starvation on the tomato. The type of growth obtained in the Cheshunt experiments resembles that noted by Nightingale *et al.* (1930) in that, in both cases, fruiting is associated with a nitrogen-starved phase, followed by the production of shoots with deep green leaves. No chemical analyses were made by Nightingale *et al.* on foliage collected during this nitrogen-starved phase, but those of Owen (1931, Table I) indicate, in each of 3 years, a low protein level of the leaves. On the other hand, it is noteworthy that in the investigations of Janssen & Bartholomew (1929), in which at the end of the experiment "very few fruits had set", and in those of Phillips *et al.* (1934), who removed all blossom-clusters as they were formed, there is no mention of a nitrogen-starved phase, while chemical analyses show a high protein level of the leaves.[2]

[1] Attention has been directed to the association of low potassium supply with protein breakdown by Richards & Templeman (1936).

[2] Sheng-Han Shih (London Univ. Ph.D. Thesis, 1936) has demonstrated that the type of growth associated with potassium starvation in cereals is affected by the level of sodium manuring. The effect of potassium deficiency on cereals in relation to the level of other nutrients, including sodium, is discussed by Gregory in a recent review (*Ann. Rev. Biochem.* Stanford Univ. 1937, pp. 557–78). It is of interest to note that potassium was replaced by sodium by Nightingale *et al.* and Phillips *et al.* but not by Janssen & Bartholomew or in the Cheshunt experiments.

The blossoms of the third flower-truss of the potassium-starved plants open at a significantly earlier date than the corresponding blossoms of the completely manured plants (Table III), although at the same period there is no difference in height (Table IV). *The acceleration of development associated with potassium starvation thus precedes reduction in growth rate.* This suggests that the high protein level to which this acceleration of development has been attributed must be a relatively direct effect of moderate potassium starvation on protein synthesis and not due merely to nitrogen accumulation associated with a reduced size of plant and unchanged rate of nitrate absorption.

The first sign of limitation of fruiting by potassium deficiency in the present experiments was failure of pollination (p. 25). Howlett (1936) records that pollen sterility in the tomato is associated with carbohydrate deficiency in contrast to nitrogen deficiency, which does not have this effect. As in the case of prolongation of the maturation period (p. 40) the effect of potassium deficiency is similar to that of carbohydrate deficiency relative to nitrogen supply. These two effects suggest that correction of the carbohydrate-nitrogen balance is an important result of potassium manuring, a view supported by the association of dark green leaves, indicative of high protein level, with the early stages of potassium deficiency, and by the acceleration of development of flower trusses from the growing point.

Owing to the deep colour and healthy appearance of the foliage of tomato plants in the early stages of potassium deficiency in association with relatively rapid vegetative development the necessity for potassium manuring to maintain fruit production is apt to be overlooked. A plant subject to the early stages of potassium deficiency under the conditions of the present experiment is relatively tall with deep green leaves, and characterized by accelerated blossoming but failure of pollination. At a more severe stage of starvation the marginal chlorosis developing into scorch in association with blotchy ripening of the fruit are distinctive diagnostic features (Bewley & White, 1926).

Summary

1. The effect of nitrogen and potassium on the fruiting of the tomato under glass is studied by observations on plants grown on completely manured plots and plots from which nitrogen and potassium respectively have been omitted from the scheme of manuring over a period of years.

2. Nitrogen starvation reduces the number of blossom-buds formed,

the percentage of buds that open and the mean numbers of fruits per flower-truss. Potassium starvation does not affect the number of blossom-buds formed or the percentage of buds that open but reduces the mean numbers of fruits per flower-truss.

3. An outstanding feature of nitrogen starvation is retardation of the rate of development of successive flower-trusses. With potassium starvation the rate of development is accelerated. Growth, estimated from increase in height of plants trimmed to a single axis by continuous removal of incipient axillary shoots, is retarded by both nitrogen and potassium starvation.

4. The "maturation period" (between opening of the blossom and ripening of the fruit) is lengthened by potassium starvation, shortened by moderate nitrogen starvation and lengthened by severe nitrogen starvation. From the present results, in conjunction with data published previously, it is concluded that the "maturation period" is shortened by a high carbohydrate-nitrogen balance and lengthened by a low carbohydrate-nitrogen balance.

5. In mid-season all plants suffer a check to growth and development demonstrated by (*a*) retardation of the rate of differentiation of leaves and flower-trusses, (*b*) cessation of growth in height, and (*c*) the occurrence of a high proportion of fruit with arrested development. This "mid-seasonal check" is accentuated by nitrogen and potassium starvation, especially the former, corresponds with the period of maximal weight of developing fruit, and is attributed to competition for nutrient supply between the fruits and vegetative parts of the plants, leading to apparent antagonism between these processes.

6. The fruiting and foliage symptoms of the potassium-starved plants are attributed to the following effects:

(*a*) The potassium in starved plants is undergoing a continuous cycle, being translocated from older parts of the plant to the growing point, incorporated in young leaves and flower trusses and subsequently re-translocated as these organs are approaching maturity.

(*b*) The level of potassium supply regulates nitrogen metabolism, a moderately low potassium supply being associated with high protein level and a greater degree of severity of potassium starvation with low protein level.

7. The results are discussed in relation to the problems of over-bearing and potassium manuring. The observed effects of potassium deficiency on fruiting (acceleration of development of flower trusses, failure of pollination and prolongation of the "maturation period" of

the fruit) are those also associated with carbohydrate deficiency relative to nitrogen supply.

In conclusion the author is indebted to Dr W. F. Bewley for permission to publish this paper and to Mr A. D. Goddard for assistance in carrying out the observations.

APPENDIX

The effect of nitrogen and potassium on order of development of leaves and flower-trusses

In the present experiments the times of opening of corresponding buds of corresponding flower-trusses have been used to estimate rates of development. Since the use of such a measure is invalidated if the treatments considered suppress the differentiation of flower-trusses or affect the number of leaves separating corresponding trusses, it is essential to consider the effect of manurial deficiency on the *order* of development of flower trusses and leaves.

In order to study the effect of manurial treatment on the position of the first-formed truss an experiment was carried out in which seed of the variety E.S.I. was divided into two batches. One batch was grown in soil known to be nitrogen-deficient. The pale yellow seedlings had stiff stems with small leaves and were clearly nitrogen-starved. The other batch was grown in the same soil enriched with a dressing of dried blood and watered periodically with nitrate. These seedlings had a high nitrogen content, as indicated by their deep green colour, succulent foliage and tendency for rapid development of incipient axillary shoots. Nevertheless, the difference in nitrogen level did not affect significantly the mean number of leaves between the cotyledons and the first truss, which was 9·5 for the nitrogen-deficient plants and 9·3 for the plants with ample nitrogen supply. Moreover, since in the present experiments the basal blossom of the first-formed truss opened within 21 days of planting, it is probable that the position of this truss had been determined before the effects of manurial deficiency became operative.

Three leaves invariably separated the flower-trusses of the main axis in the present experiment with the exception of the intervals between the first and second and second and third trusses, which consisted of five leaves in a variable percentage of plants. This percentage differs from season to season but these differences, shown in Table XI, cannot be related to variation in manurial treatment. The largest difference in

comparison with the completely manured plants is shown by the nitrogen-starved plants for the leaf interval between the second and third trusses, for 15% more of these plants possess five or more leaves instead of the three or four leaves characteristic of the completely manured plants. This difference is not sufficiently consistent to be considered significant ($P=0{\cdot}15$). The assumption may, therefore, justifiably be made that differences in times of opening of corresponding buds of different trusses are due to the effects of manurial treatment on the rate of development of the growing point.

Table XI

Relative effect of manurial deficiency on number of leaves developing between flower-trusses

(The percentages of plants with an even number of leaves are mainly due to abnormality such as the production of opposite leaves)

Between first and second flower-trusses

	3				4			
	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	61	33	43	53	7	8	10	17
Nitrogen-starved	82	25	67	79	—	4	—	4
Potassium-starved	92	25	50	46	—	4	—	8

	5				6			
	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	32	59	43	30	—	—	4	—
Nitrogen-starved	14	59	29	13	4	12	4	4
Potassium-starved	8	71	38	42	—	—	12	4

Between second and third flower-trusses

	0	3				4			
	1937	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	—	65	92	37	60	14	4	3	10
Nitrogen-starved	4	82	88	29	46	—	—	—	4
Potassium-starved	—	66	96	46	46	17	4	—	8

	5				6				7			
	1933	1935	1936	1937	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	21	4	53	30	—	—	7	-	—	—	—	–
Nitrogen-starved	18	12	50	46	—	—	17	—	—	—	4	—
Potassium-starved	17	—	37	46	—	—	17	—	—	—	—	—

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THE EFFECT OF MANURING UPON APPLE FRUITS

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(With 2 Text-figures)

CONTENTS

I. Introduction

Since 1923 one of us has investigated the control of apple scab (*Venturia inaequalis* Aderh.) in Northern Ireland orchards (Northern Ireland, 1935: Muskett & Turner, 1929) by the adoption of routine summer spraying. During the course of this work the carrying out of the same summer-spraying programme (Northern Ireland, 1927) gave consistently better results in some orchards than in others. In one particular orchard, where the measure of control obtained was usually low, it was decided to investigate whether this could be explained in any way in terms of tree nutrition and also to make observations upon the effect of manuring upon the fruit. In some ways this orchard which was situated at Dunadry, Co. Antrim, was ideal for the work. It was a well-kept grass orchard and comprised 151 trees, all of which, with one exception (Lane's Prince Albert), were of the variety Bramley's Seedling. The trees were all of the same age (20 years old in 1929), had been worked on the same stock and showed little variation in size. They were widely spaced, having been planted 20 ft. apart, and the general conditions were favourable for the carrying out of a manurial experiment. On the other hand, the orchard was in an exposed position and very susceptible to blossom damage by spring frosts. It was inclined to crop erratically and could not be regarded as entirely satisfactory from this point of view. Owing to repeated damage to the blossom by spring frosts satisfactory results from this investigation over a period of years were not obtained but, so striking and clear-cut were the results obtained in the one year (1930) in which the orchard cropped well, that it has been decided to put them on record.

II. Soil analysis

An analysis of the soil of the Dunadry orchard was made by Mr J. C. Baird, of the Department of Agricultural Chemistry, Queen's University of Belfast. The results are given in Table I. From these data it will be seen that the soil is very deficient in nitrogen, but that the supplies of potash

Table I

Analysis of soil from Dunadry orchard

Chemical analysis		Mechanical analysis	
Moisture	6·52	Coarse sand	16·70
Nitrogen	0·231	Fine sand	24·50
Phosphoric acid (total)	0·114	Silt	21·65
Potash (total)	0·411	Clay	24·70
Phosphoric acid (available)	0·043	Loss on ignition (organic matter)	11·28
Potash (available)	0·024	Carbonates	0·044

and phosphates, as regards both total content and available material, are ample. The ground flora of the orchard for some seasons past had indicated the soil to be deficient in nitrogen.

III. Spraying programme

During the period under review the normal practice in orchards in Ulster where trees of the variety Bramley's Seedling were grown and which were not subject to attack by apple capsids was to spray the trees with a tar distillate wash in late winter for the control of apple sucker and aphides, and to follow this up with at least three summer sprayings with Bordeaux mixture for the control of apple scab (Northern Ireland,

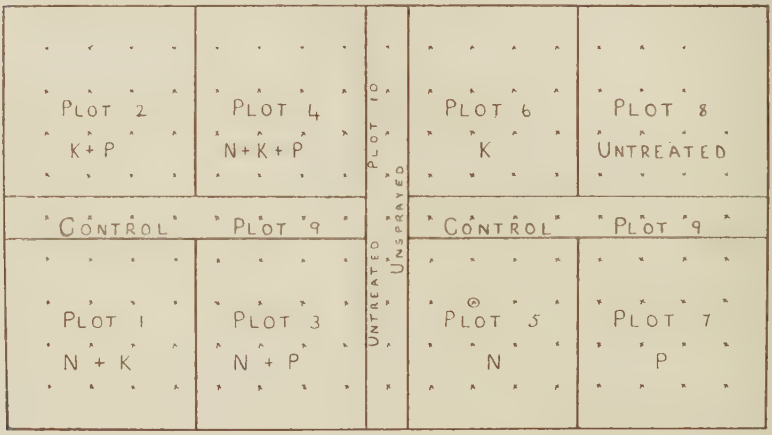


Fig. 1. The lay-out. 1929-30. × position of tree of variety Bramley's Seedling; ⊗ position of tree of variety Lane's Prince Albert.

1927). During the course of this investigation the trees, with the exception of those in plot 10 (see Fig. 1), were so treated, the only alteration being that, in 1929, no application of a tar distillate was given. The trees in plot 10, which had been used as controls during previous spraying experiments, were neither sprayed nor manured.

IV. The lay-out

In 1929 the orchard was divided into ten plots as shown in Fig. 1. Each plot with the exception of Nos. 8 and 10 contained sixteen trees of the variety Bramley's Seedling. The odd tree of the variety Lane's Prince Albert was situated in plot 5. All the plots with the exception of Nos. 8, 9 and 10 received manurial treatment. Of the unmanured

plots No. 9 represents the true control, since the trees here received the same spraying programme and similar cultural treatment to those in the manured plots. Plot 8 had been utilized for the growing of potatoes before the commencement of the experiment. Guard rows were not provided between trees in plots receiving different treatments, because of the wide planting system adopted. This lay-out was changed in 1931.

V. Manurial treatment

The effects of manuring the trees with nitrogen, potash and phosphate, applied singly or in combination, were investigated, and the following fertilizers were employed:

Sulphate of ammonia	(20·6% N)
Muriate (chloride) of potash	(50·0% K_2O)
Superphosphate	(16·03% soluble P_2O_5)

It was decided that more pronounced results would be likely to ensue from the application of larger quantities of fertilizers than those generally used and, therefore, the manures were applied as follows: sulphate of ammonia, 10 lb. per tree; muriate of potash, 5 lb. per tree; and superphosphate, 10 lb. per tree. In 1931 and 1932 certain plots received double the usual dressing of muriate of potash or superphosphate. Farmyard manure was applied in 1931 and 1932 to one plot at the rate of 10 cwt. per tree). The fertilizers were broadcast evenly around each tree and extending beyond the spread of the branches. Care was taken that none was applied within a yard of the trunk. The potash and phosphate were applied during the middle of March and the nitrogen towards the end of May.

VI. Experimental methods

At the time of picking the fruit the produce of each tree was divided into two classes, (i) apples which showed any signs of established infection by scab, and (ii) those which showed no signs of such infection. Each class was weighed separately and the weights recorded for each tree. From these figures the percentage weight of scabbed fruit borne by each tree was calculated.

The resistance of the fruit to invasion by *Cytosporina ludibunda* strain CE was determined by the method of Gregory & Horne (1928) on samples of apples selected at random in the orchard from the produce of each plot and despatched to the Imperial College, London, where the tests were carried out. Apples were inoculated on one side with the fungus and stored at laboratory temperatures until the rotted tissue was

estimated, and the radial advance of the fungus, in mm. per day, for each individual apple was determined. From these data the average mean radial advance per day was calculated for each sample.

The nitrogen content of the fruit was determined, using samples selected at random from each plot, by the Kjeldahl method according to the technique described by Archbold (1925).

The determination of the radial spread of *C. badibanda* CE in sterilized apple pulp was made by preparing pulp from each of the various samples. The pulp was sterilized by steaming and used as a medium without the addition of any other ingredient. Plates were prepared in triplicate, and the pulp from apples of each sample inoculated with the fungus, incubated at a constant temperature and the growth of the fungus measured after 11 days.

VII. Results for 1929

During the summer of 1929 it was possible to pick out all the trees which had received a dressing of sulphate of ammonia alone or in combination with other fertilizers, since the trees receiving nitrogenous manure bore large dark green-coloured leaves. Manuring with potash or phosphate was observed to have no effect on the foliage colour. The crop in 1929 was very poor, owing to blossom damage by spring frosts, and although some colour differences were noted in the fruit no further observations were made for this year.

VIII. Results for 1930

Field observations

Apart from the darker foliage difference, it was clearly shown in 1930 that nitrogenous manuring increased the amount of blossom produced by the trees, those in the plot receiving complete manuring being adjudged the best. Furthermore, the trees receiving nitrogenous dressings were in bloom about 10 days earlier than those which were untreated or received no nitrogen. This precocious and profuse blossoming was not, however, reflected in the fruit yields (see Table II).

The trees in the plots receiving nitrogen alone or in combination with other fertilizers showed increased growth in 1930.

The crop was good for the year, and the fruit from the various plots showed striking differences in colour and texture. The fruit from the trees which had received nitrogen, alone or in combination with other fertilizers, was very green in colour and soft in texture, while that from the control, the potash and the phosphate-treated plots was highly

coloured and very hard. Little difference could be seen between plots manured with potash and with phosphate, but phosphates appeared to produce a pleasant yellowness of the skin of the fruit.

Table II shows the mean values for total yield and percentage weight of scabbed fruit from each plot. These values are each based on records of fifteen trees. The mean value for yield per tree for the nine trees in plot 10 which was untreated and unsprayed was 3·3 lb., two of the trees bearing no crop. The mean percentage weight of scabbed fruit borne by each tree which cropped in plot 10 was 61·5.

Observations on resistance to fungal invasion

A sample of forty apples from each of nine plots was available for the determination of resistance to fungal invasion by the method of radial advance. The apples were inoculated from 17 to 19 December and estimations of rotted tissue made from 5 to 7 January. Samples of ten apples from each of the nine plots were available for analysis for nitrogen content. The results of these two experiments together with the rate of radial spread of *C. ludibunda* in sterilized apple pulp from each of the samples are given in Table II and expressed graphically in Fig. 2.

Table II

Effect of varied manurial treatment, 1930

No. of plot	Treatment	Mean yield lb.	Mean % weight of scabbed fruit	Average mean radial advance (mm. per day)	Radial spread in apple pulp (mm. in 11 days)	Nitrogen-content (% of fresh weight)
6	K	67·5	15·0	0·082	13·7	0·0185
2	K + P	53·1	8·0	0·118	15·7	0·0233
8*	X*	91·1	10·5	0·130	16·2	0·0254
7	P	71·0	6·2	0·150	16·0	0·0256
9	C	58·6	5·4	0·259	16·5	0·0218
4	N + K + P	94·5	38·7	0·456	26·7	0·0584
3	N + P	58·4	43·7	0·678	29·0	0·0465
5	N	68·1	32·1	1·213	29·0	0·0534
1	N + K	25·7	26·2	1·249	31·5	0·0619

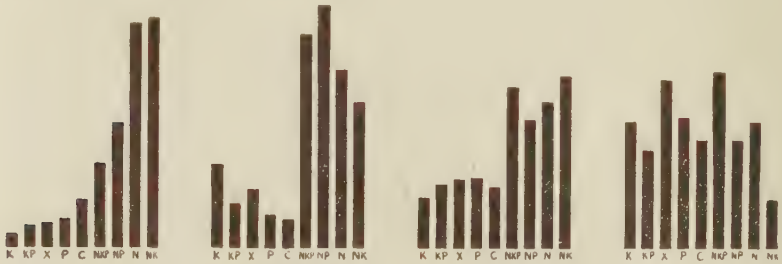
K = muriate of potash; P = superphosphate; C = control; N = nitrogen; X = untreated but does not constitute a control plot.

* This plot contained only fourteen trees and for reasons previously stated was not comparable with other plots. Although not receiving manurial treatment it does not constitute a true control.

It will be seen from Table II that as regards percentage weight of scabbed fruit, radial advance and nitrogen content, the plots fall very clearly into two main classes, (i) those receiving no nitrogen, and (ii) those receiving nitrogen. It is also clear that nitrogenous manuring

lowered the resistance of the fruit to fungal invasion, increased the amount of scabbed fruit and raised the nitrogen content of the fruit (see Fig. 2 A, B and C). The values for nitrogen content run almost parallel with those for radial advance, low nitrogen content being associated with high resistance and vice versa.

It is also interesting to note that the radial spread of *C. ludibunda* in sterilized apple pulp runs almost parallel with the values for radial advance calculated for living tissue.



A Radial advance B Percentage weight of scabbed fruit C Nitrogen-content D Yield

Fig. 2. Effect of varied manurial treatment, 1930.

Statistical analysis of results

A. *Correlations.*

For the purpose of calculating the correlation between the mean values of radial advance and for percentage weight of scabbed fruit the values given for the nine plots in Table II were employed. As has been previously stated, the percentage weight of scabbed fruit was calculated for individual trees from the total yields. Variations in yield were considerable, ranging from 5 to 176 lb. The following result was obtained:

$$r_{SR} = \pm 0{\cdot}6395$$

R = radial advance per day, S = percentage weight of scabbed fruit. Reference to Fisher's Table V A (Fisher, 1934) shows that the value obtained falls below the 5% probability value (0·6664). The correlation is therefore high but not significant. It must be remembered, however, that only nine pairs of values entered into the calculation. The result suggests that fruit which is resistant to an organism which is responsible for causing decay in storage is also resistant to the attacks of the scab organism during growth. It should be pointed out in support of this opinion that the scab lesions were most numerous and largest on apples

from plots which received nitrogenous manure and showed high radial advance.

The rate of radial advance and the percentage weight of scabbed fruit have both been correlated with nitrogen content of the fruit (Table II) with the following results:

$$r_{RN} = +0{\cdot}8571, \quad r_{SN} = +0{\cdot}8292.$$

N = percentage nitrogen in fresh weight of apples. Both these correlations are significant since the value of the coefficient obtained in each case exceeds the 1 % probability value (0·7977).

The coefficient of correlation between the values for mean radial advance and for radial spread of the fungus on sterilized pulp (Table II) has been calculated. The result obtained was as follows:

$$r_{RM} = +0{\cdot}9112,$$

M = radial spread of fungus on sterilized pulp. This correlation is highly significant since the value obtained exceeds the 1 % point (0·7977) and the result indicates that the radial advance of *C. ludibunda* in living apple tissue is directly correlated with the radial spread on sterilized pulp.

B. *Analysis of variance and calculation of standard error.*

Radial advance.

For the purpose of analysis of variance (Fisher, 1934) there were available nine samples, each consisting of forty apples, giving 8 degrees of freedom for treatment and 351 for error. The result of the analysis is given in Table III. The value of Z (2·0754) is more than four times the 1 % probability value and hence is highly significant. This result shows quite definitely that manurial treatment has had a very real effect on resistance of the fruit to invasion by the fungus employed.

Table III

Analysis of variance. Effect of manurial treatment on radial advance, 1930.

	Degrees of freedom	Sum of Squares	S.D.	$\log_e$S.D.	Z	1 % probability value
Treatment	8	69·52380	2·94	+1·07841	2·0754	0·4604
Error	351	47·97815	0·369	−0·99695	—	—

In order to determine which particular treatments were responsible for the high value of Z, the standard errors for the differences of mean values were calculated. These were not based upon the standard deviation for error given in the analysis of variance (Table III), because it was found that the variance within treatment greatly increased with the

presence of nitrogen as a manurial constituent. Instead, the standard error in each case was calculated from the standard deviation found for the samples compared. The values obtained were as follows:

K *v.* K + P	− 0·036 ± 0·011	X *v.* N + P	− 0·548 ± 0·086
K *v.* X	− 0·048 ± 0·010	X *v.* N	− 1·083 ± 0·092
K *v.* P	− 0·068 ± 0·010	X *v.* N + K	− 1·119 ± 0·105
K *v.* C	− 0·177 ± 0·015	P *v.* C	− 0·109 ± 0·015
K *v.* N + K + P	− 0·374 ± 0·053	P *v.* N + K + P	− 0·306 ± 0·054
K *v.* N + P	− 0·596 ± 0·089	P *v.* N + P	− 0·528 ± 0·089
K *v.* N	− 1·131 ± 0·092	P *v.* N	− 1·063 ± 0·092
K *v.* N + K	− 1·167 ± 0·105	P *v.* N + K	− 1·099 ± 0·105
K + P *v.* X	− 0·012 ± 0·011	C *v.* N + K + P	− 0·197 ± 0·054
K + P *v.* P	− 0·032 ± 0·012	C *v.* N + P	− 0·419 ± 0·089
K + P *v.* C	− 0·141 ± 0·105	C *v.* N	− 0·954 ± 0·093
K + P *v.* N + K + P	− 0·338 ± 0·054	C *v.* N + K	− 0·990 ± 0·105
K + P *v.* N + P	− 0·560 ± 0·088	N + K + P *v.* N + P	− 0·222 ± 0·103
K + P *v.* N	− 1·095 ± 0·092	N + K + P *v.* N	− 0·757 ± 0·106
K + P *v.* N + K	− 1·131 ± 0·105	N + K + P *v.* N + K	− 0·793 ± 0·117
X *v.* P	− 0·020 ± 0·011	N + P *v.* N	− 0·535 ± 0·127
X *v.* C	− 0·129 ± 0·015	N + P *v.* N + K	− 0·571 ± 0·137
X *v.* N + K + P	− 0·326 ± 0·053	N *v.* N + K	− 0·036 ± 0·139

The degree of significance of the difference between various pairs of means is given in Table IV, where the figures in the columns represent the difference between various pairs of mean values divided by the standard error of the difference. In Table IV differences which exceed twice the standard error are regarded as significant. It should be noted that as compared with the control plot nitrogenous manuring has in every case significantly reduced the resistance of the fruit to fungal invasion, while the application of mineral manures without nitrogen has in all cases significantly increased the resistance above that of the control plot.

Table IV

Degree of significance of difference between pairs of mean values for radial advance for samples from plots, 1930

(Figures represent the difference between pairs of mean values divided by the S.E. of the difference.)

	K	K + P	X*	P	C	N + K + P	N + P	N
K + P	**3**							
X*	**4**	1						
P	**6**	**2**	1					
C	**12**	**9**	**8**	**7**				
N + K + P	**7**	**6**	**6**	**5**	**3**			
N + P	**6**	**6**	**6**	**6**	**4**	**2**		
N	**12**	**11**	**11**	**11**	**10**	**7**	**4**	
N + K	**11**	**10**	**10**	**10**	**9**	**6**	**4**	0

* Plot untreated but does not constitute a true control.

Percentage weight of scabbed fruit.

Although it is uncertain whether the application of analysis of variance is strictly valid in the case where estimates of variance are

based on percentage values obtained from uneven yields, nevertheless an analysis has been attempted. For the purpose of the analysis there are eight treatments, plot 8 (see Fig. 2) being eliminated because it contained only fourteen trees. For treatment there were 7 degrees of freedom. Since there were fifteen trees under each treatment error gave 112 degrees of freedom. For error the variance within plots for percentage weights of scabbed fruit calculated for individual trees was determined. The result obtained was as follows:

Table V

Analysis of variance. Effect of manurial treatment on percentage weight of scabbed fruit, 1930.

	Degrees of freedom	Sum of Squares	S.D.	$\log_e$S.D.	Z	1% probability value
Treatment	7	24593·55	59·2	4·08092	1·4347	0·4878
Error	112	22316·64	14·1	2·64617	—	—

The value of Z greatly exceeds the 1% probability value, suggesting that there are real differences in the degree of scab attack between plots receiving different treatments.

The standard errors for the differences between treated plots and the control plot were calculated and these were based on the S.D. found for each pair of samples compared. This method was followed because high variance was associated with nitrogenous manuring and high mean percentage weights of scabbed fruit, whereas low variance was associated with non-nitrogenous or no manuring and low percentage weights of scabbed fruit. The differences between the mean values for treated plots and the control plot are given in Table VI, together with the requisite standard errors. Differences which exceed twice the standard error may be regarded as significant.

Table VI

Differences of mean values for percentage weight of scabbed fruit, 1930

Treatment	Difference from control plot
K	+ 9·6 ±3·1
K + P	+ 2·6 ±1·5
P	+ 0·8 ±1·1
N + K + P	+ 33·3 ±3·7
N + P	+ 38·3 ±5·1
N	+ 26·7 ±4·6
N + K	+ 20·8 ±5·8

The results suggest that five plots including four which received nitrogenous manuring show a significantly higher percentage weight of

fruit attacked by scab than the control plot. In general this result bears out those obtained by the method of correlation.

IX. Results for 1931 and 1932

Field observations

The observations made during 1929 and 1930 on the effects of nitrogen on flowering, foliage and growth of the trees were confirmed. Apples from trees which received nitrogenous manuring were green in colour, and those from trees which received no nitrogenous manure were blushed but the distinctions were not so clear as in 1930.

The yield of fruit in both years was very small and, although the crop borne by each tree and the percentage showing established infection with *Venturia inaequalis* were recorded, it is considered that no useful purpose would be served by a statement of results.

Observations on resistance to fungal invasion

In 1931 a sample of fifteen apples from each of the thirty plots into which the orchard was divided in that year were available for biological work. These were inoculated 23–27 November and estimations of the rotted tissue were carried out 21 days from the time of inoculation. Samples of six to fifteen apples from each of these plots were analysed for nitrogen content in 1931. The results of these experiments are given in Table VII. It should be observed that in most cases plots which received nitrogenous manuring in either 1930 or 1931 gave high values for nitrogen content and radial advance.

In 1932 a sample of twenty apples was selected to represent the population of apples produced by trees which had received nitrogenous manuring either alone or in addition to other fertilizers, and another sample of equal number to represent the population of apples borne by trees which had received no nitrogenous manure, but some of which had received a dressing of potash or phosphate. Only plots which had received exactly the same treatment since the commencement of the experiment in 1929 were represented in the samples. The apples were inoculated on 10 January. The data for average mean radial advance for these samples are given in Table VIII side by side with the average means of comparable samples inoculated in 1930 and 1931. It should be noted that in all three years the radial advance is much higher for samples from plots receiving nitrogenous manuring than from plots which did not receive nitrogen.

Table VII

Effect of varied manurial treatment on apple fruit, 1931

Plot		Manurial treatment 1929 and 1930	Manurial treatment 1931	Average mean radial advance (mm. per day)	Nitrogen content % fresh weight
Block I	1	N	—	0·370	0·0444
	2	N	N	0·551	0·0710
	3	N	2P	1·027	0·0719
	4	N	2K	0·365	0·0550
Block II	5	K	—	0·610	0·0507
	6	K	K	0·553	0·0579
	7	K	N	0·802	0·0603
	8	K	2P	0·094	0·0279
Block III	9	P	—	0·239	0·0380
	10	P	P	0·173	0·0424
	11	P	2K	0·181	0·0402
	12	P	N	0·789	0·0493
Block IV	13	N + K	—	0·401	0·0633
	14	N + K	N + K	1·168	0·0835
	15	N + K	2P	0·808	0·0562
	16	N + K	2K	0·655	0·0697
Block V	17	N + P	—	0·326	0·0432
	18	N + P	N + P	1·292	0·0550
	19	N + P	2K	1·380	0·0516
	20	N + P	2P	1·135	0·0705
Block VI	21	K + P	—	0·152	0·0439
	22	K + P	K + P	0·388	0·0374
	23	K + P	2K	0·214	0·0388
	24	K + P	N	0·741	0·0540
Block VII	25	N + K + P	—	0·824	0·0723
	26	N + K + P	N + K + P	1·303	0·0885
	27	N + K + P	2P	1·092	0·0601
	28	N + K + P	2K	0·580	0·0643
	29	Control	Control	0·210	0·0343
	30	Control	F.Y.M.	0·150	0·0367

Table VIII

Average mean radial advance (mm. per day), 1930–2

Year	No. of apples in each sample	No. of trees represented by each sample	Average mean radial advance (mm. per day) for sample from trees receiving nitrogenous manuring	Average mean radial advance (mm. per day) for sample from trees receiving no nitrogenous manuring	Difference
1930	160	64	0·889	0·153	**0·736** ±0·051
1931	60	20	1·08	0·33	**0·75** ±0·095
1932	20	20	0·89	0·54	**0·35** ±0·110

Statistical analysis of results

It has been possible to make only one correlation, viz. that between radial advance and nitrogen content of the fruit using the values given in Table VII. The result was as follows:

$$r_{RN} = +0{\cdot}7277.$$

This correlation is significant since the value obtained greatly exceeds the 1 % probability value (0·4641).

Analysis of variance was applied to the data of radial advance for samples from plots each of which received the same treatment from year to year during the experiment. For this purpose there were available eight samples each consisting of fifteen apples, giving 7 degrees of freedom for treatment, and 112 for error. The result of the analysis is given in Table IX. The value of Z greatly exceeds the 1% probability value, again indicating that manuring has exercised a real effect on the resistance of the fruit to fungal invasion.

Table IX

Analysis of variance. Effect of manurial treatment on radial advance, 1931.

	Degrees of freedom	Sum of Squares	S.D.	$\log_e$S.D.	Z	1% probability value
Treatment	7	23·8785	1·84	+0·60977	1·3863	0·4878
Error	112	23·7272	0·46	−0·77652	—	—

The standard errors for the differences in radial advance between each treated plot dealt with in the analysis of variance and the control plot, based on the standard deviation estimated for the requisite pairs of samples are as follows: C *v.* P, $+0{\cdot}037 \pm 0{\cdot}106$; C *v.* K+P, $-0{\cdot}178 \pm 0{\cdot}164$; C *v.* N, $-0{\cdot}341 \pm 0{\cdot}176$; C *v.* K, $\mathbf{-0{\cdot}343} \pm 0{\cdot}167$; C *v.* N+K, $\mathbf{-0{\cdot}958} \pm 0{\cdot}170$; C *v.* N+P, $\mathbf{-1{\cdot}082} \pm 0{\cdot}152$ and C *v.* N+K+P, $\mathbf{-1{\cdot}093} \pm 0{\cdot}162$. It is seen that the high value of Z is mainly conditioned by the three treatments N+K, N+P and N+K+P which gave fruit of very low resistance.

Similar calculations were made for plots which were untreated in 1931 but which had been treated in 1929 and 1930 with the following results: C *v.* K+P, $+0{\cdot}058 \pm 0{\cdot}104$; C *v.* P, $-0{\cdot}029 \pm 0{\cdot}129$; C *v.* N+P, $-0{\cdot}116 \pm 0{\cdot}150$; C *v.* N, $-0{\cdot}160 \pm 0{\cdot}129$; C *v.* N+K, $-0{\cdot}191 \pm 0{\cdot}149$; C *v.* K, $\mathbf{-0{\cdot}400} \pm 0{\cdot}180$ and C *v.* N+K+P, $\mathbf{-0{\cdot}614} \pm 0{\cdot}182$. In this case only two differences are significant, viz. those where K and N+K+P were used as fertilizers in 1929 and 1930.

It must be remembered that the results for 1931 given above are based on fruit in a year when the yield was negligible from a commercial point of view.

X. Discussion

This investigation was primarily undertaken with the object of determining the effect of manurial treatment upon the incidence of apple scab, but its scope was subsequently extended so as to embrace a study of the effects of treatment upon resistance of the fruit to fungal invasion

as measured by the radial advance of *C. ludibunda*, as well as observations upon growth, yield, bloom, fruit colour and nitrogen content.

Wallace (1933) has shown by pot experiments that the omission of nitrogen from nutrient solutions supplied to apple trees brings about a delay in the opening of blossom buds, a reduction of blossom formation, a reduction in the amount of foliage and also leads to the production of fruits which are often highly coloured or of chlorotic appearance. These observations have been confirmed by the present investigation where it was known that the soil was deficient in nitrogen, and it was further shown that by nitrogenous manuring such conditions could be rectified.

Unfortunately a good crop was obtained only in one year (1930), and since in the other three years very poor crops were produced the greater part of the results presented here are for one year only. Studies in the resistance of the fruit to fungal invasion were, however, carried out during three years, but during one year (1932) the samples available were very small.

The results in 1930 suggest that the fruit from the trees receiving nitrogen yielded a definitely higher percentage weight of apples attacked by scab than those receiving no nitrogen. The true position as regards incidence of scab is not quite reflected by the figures in Table II, since the scab lesions on apples from trees receiving no nitrogen were small; those on fruit from trees receiving nitrogen being larger and more numerous. Potash manuring may also be responsible for an increase in the percentage weight of fruit showing established infection by scab. This result obtained by potash manuring points in the same direction as that obtained by Grubb (1930) at East Malling.

Manurial treatment has been shown to exercise a great effect on the resistance of the fruit to fungal invasion by *C. ludibunda*. In 1930 it was found that the mean rate of growth of the fungus in the samples from plots receiving nitrogenous manuring was in every case much higher than in apples from plots which received non-nitrogenous or no manure. For example, the mean growth rate in the sample from the plot treated with both nitrogen and potash was actually more than fifteen times that in the sample from the plot which received potash only, and almost five times that in the sample from the control plot. In 1931 and 1932 when the crops borne were very small it is interesting to note that even under these conditions and where only small samples for each treatment were available it was again possible to demonstrate that nitrogenous manure lowered the resistance of the fruit.

The application of potash, phosphate, or of both brought about a

definite reduction in the rate of radial advance in 1930. The mean rate in the sample from the control plot was more than three times that in the apples from the plot treated with potash. The effect of manuring with potash or phosphate obtained in 1930 was not repeated in 1931. Since the samples available in the latter year were small it is doubtful whether any real confidence can be placed in the fact that in 1931 treatment with potash appeared to lower the resistance. However, it has been shown in another investigation (Horne, 1935-6) that manuring with potash in one year lowered resistance and in the following year increased the resistance of the apples borne on the same trees. In 1932 the samples available were so small that only the effect of nitrogenous as opposed to non-nitrogenous or no manuring was investigated.

Great confidence can be placed in the results for radial advance obtained in 1930 because in that year the yield was high and large samples were available for experimental work. Further, it has been shown that the values obtained in that year for the rate of radial spread of *C. ludibunda* in sterilized pulp from each of the samples ran parallel with the values for its rate of radial advance in living tissues. This close relationship between the growth rates in dead and living tissues indicates that the rate of advance of the fungus in the living fruit is governed largely by the chemical composition of the fruit. This is in agreement with the work of Seth (1934) who showed that the rate of spread of the fungus on artificial medium is influenced by the chemical composition of the medium.

The only information available with regard to the effect of manuring on chemical composition of the fruit is that for nitrogen content. This shows that nitrogenous manuring greatly increases the nitrogen content, and it has been proved that in each of the two years for which the values are available that nitrogen content and radial advance are directly correlated.

The suggestion of a marked relationship in 1930 between radial advance and percentage weight of scabbed fruit is of interest. In another orchard in Northern Ireland it has since been shown that there was in one year a high but not significant correlation between radial advance and percentage weight of scabbed fruit (unpublished data).

It appears from the results presented that the nitrogen content of the fruit varies in different seasons and that the higher the yield the lower the nitrogen content. The relation between yield and nitrogen content is reflected in the rate of fungal invasion; low yields being associated with low resistance and high nitrogen content, and high yields

with high resistance and low nitrogen content. Further work along these lines is desirable and although these observations hold for the experimental orchard and for the years under review, it is uncertain whether or not they are of general application.

During the investigation it was observed in all three years that, when the figures for radial advance were studied, high variance within samples was associated with nitrogenous manuring, whereas low variance was associated with non-nitrogenous or no manuring. It may be suggested that high variance in the samples from plots heavily manured with nitrogen in a readily available form may be due, to some extent, to the rapid and uneven absorption of the nutrient by the tree, although the manure was spread as evenly as possible.

Wallace (1933) has pointed out that nitrogen starvation is of frequent occurrence under grass systems of orchard cultivation. The present work demonstrates that in rectifying this condition care must be exercised in the application of nitrogenous fertilizers or the quality of the fruit may suffer very severely; the results also suggest that such manuring may render the control of scab more difficult. It has been indicated also that under Northern Ireland conditions the carrying out regularly of an efficient spraying programme for the control of diseases and pests is a very important factor in the production of good crops.

XI. Summary

1. Manurial treatment exercises a strong influence on the growth of apple trees, their time of blossoming, the amount of bloom, the foliage colour and the fruit colour. The effect is mainly due to nitrogen which induces increased growth, earlier flowering, more bloom, greener foliage and softer and greener fruit. Potash and phosphate have little effect on the growth of the trees, or on blossoming or foliage, but favour the production of more highly coloured fruit.

2. Nitrogenous manuring increases the nitrogen content of the fruit. Thus in 1930 the mean nitrogen content for the samples from plots receiving and not receiving nitrogen, was 0·0229 and 0·0550 respectively. In the following year the results for similarly treated plots were 0·0430 and 0·0745 respectively. Considering the results obtained in the years in question the mean nitrogen content for 1930 when there was a good yield was 0·0372 in contrast with 0·0587 for 1931 when the yield was poor. The control plot alone gave values of 0·0218 and 0·0343.

3. In 1930 the rates of radial advance (in mm. per day) of *Cytosporina ludibunda* for samples from plots receiving different treatments were

K, 0·082; K+P, 0·118; X, 0·130; P, 0·150; C, 0·259; N+K+P, 0·456; N+P, 0·678; N, 1·213; and N+K, 1·249. The rate for the sample from the plot treated with N+K is fifteen times that calculated for the apples from the plot treated with K. The effect of nitrogenous as compared with non-nitrogenous or no manuring has been studied in each of three years. The mean values for radial advance obtained for samples from plots receiving no nitrogen were 1930, 0·153; 1931, 0·33; 1932, 0·54, while for samples from plots receiving nitrogenous manuring the values were 1930, 0·889; 1931, 1·08; and 1932, 0·89. The values obtained in 1930 receive confirmation from observations on the radial spread of the fungus on the sterilized tissue from apples produced on trees in the various plots. The value $r_{RM} = +0{\cdot}9112$ was obtained for the correlation between radial advance (R) and radial spread (M). In 1930 the rate of growth of the fungus in the sample from the plot treated with K was one-third of that in the sample from the control plot.

4. Radial advance is correlated with nitrogen content (N) of the fruit and this relationship is shown by the following values for the correlation coefficient: 1930, $r_{RN} + = 0{\cdot}8571$; 1931, $r_{RN} = +0{\cdot}7277$.

5. Nitrogenous manuring increases the intensity of the attack of *Venturia inaequalis* on the fruit in the orchard. Evidence is produced in support of the suggestion that fruit resistant to attack by *V. inaequalis* may also be resistant to invasion by a rot producing fungus in storage. The value of the correlation between radial advance (R) and the percentage weight of scabbed fruit (S) was $r_{RS} + = 0{\cdot}6395$. The percentage weight of scabbed fruit was shown to be correlated with the nitrogen content of the fruit and the value of the coefficient obtained was $r_{SN} = +0{\cdot}8292$.

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STUDIES IN POTATO STORAGE

II. INFLUENCE OF (1) THE STAGE OF MATURITY OF THE TUBERS AND (2) THE STORAGE TEMPERATURE FOR A BRIEF DURATION IMMEDIATELY AFTER DIGGING, ON PHYSIOLOGICAL LOSSES IN WEIGHT OF POTATOES DURING STORAGE

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(With 3 Text-figures)

In paper I (Singh & Mathur, 1937) of this series attention was given to the problem of maturity in potatoes, and it was shown that, while developing on the vine, the potato tuber passes through three more or less well-defined stages designated as adolescence, maturity, and ripening. In extending the work particular attention was given to a study of the influence of developmental stage of the tubers at the time of harvest on physiological losses during storage under different conditions. Information was sought regarding the extent to which the chief maturing processes in the tubers might continue in storage. Will potatoes, immature yet large enough for seed, attain during storage the percentage composition and the physiological condition characteristic of the tubers allowed fully to mature on the vine?

At the time of harvest the periderm layer of the tuber is only partly formed and the process of periderm formation continues for some time during storage, depending upon environmental conditions. Several investigators have studied the anatomy of the periderm of potato and the conditions conducive to rapid cork formation. They generally agree that the periderm formation is hastened by high temperature and high humidity. It is desirable to hasten the process of periderm formation in order to reduce the loss in weight due to increased transpiration and respiration during storage. Since the periderm layer when completely formed is impermeable to water and gases it might be inferred, as indeed it has been suggested by several investigators, that the total carbon dioxide production during metabolism will not be the same as its

superficial evolution, and the internal atmospheres of tubers will, in consequence, be characterized by fairly high concentrations of carbon dioxide.

A study of the storage-room atmosphere surrounding the bin of potatoes is likely to throw light on such questions as limiting the size of the bins, as well as the number and duration of aerations that are necessary for successful storage. Results of potato-storage experiments in which small lots of tubers have been stored may be of little value in solving the problems in the storage house where the potatoes are usually stored in bulk. A small lot of tubers exposed to the air on all sides will have an entirely different environment and will not react with regard to its surroundings in the same way as will those stored in bulk.

Experimental procedure

The experimental crop was raised from seed belonging to the variety Farrukhabad. During 1934–5, beginning when the vines were in bloom, four to six typical hills were dug at intervals of 3 days and the tubers divided into three lots, each consisting of an equal number of tubers of approximately the same size. One lot was sampled immediately for respiration measurements and chemical analysis (the results have already been presented in the first paper (Singh & Mathur, 1937), while the other two were placed in temporary storage at two fairly constant temperatures of 7 and 18° C. for 10–12 days. At the expiry of the preliminary storage periods, the two lots of tubers were placed in the same permanent storage at $13 \pm 0{\cdot}9°$ C. The storage lots were all taken out for experimentation on 25 May 1935.

Determinations of respiration intensity and chemical constituents were made according to methods already given (Singh & Mathur, 1937).

Results

Fig. 1 shows that, in general, the tubers that had been in a temporary storage at 7° C. before being permanently stored at 13° C. possess a higher respiration intensity than the ones pre-stored at 18° C. It was also observed that the tubers pre-stored at 7° C. terminated their period of dormancy sooner and exhibited a more extensive sprout growth than the comparable lots stored at the higher temperature for a brief duration. This fact probably accounts for the greater respiration rate of the lot pre-stored at 7° C.

It is evident from Tables I and II that at the end of the storage period the lots pre-stored at 18° C. possess in general a higher percentage of

total sugars than those temporarily stored at 7° C. The greatest loss in the total sugar content occurred in the adolescent tubers, although they still contained the highest percentage of total sugars at the end of the storage period. In contrast to total sugar content the quantity of reducing sugars increases during storage in adolescent tubers under both

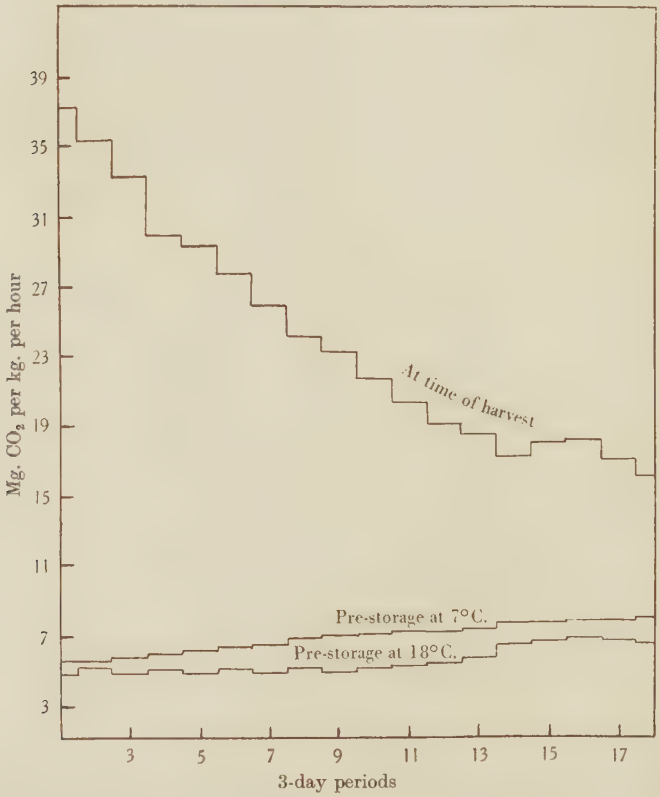

Fig. 1. Respiration intensity of potatoes of different developmental stages at time of harvest and at the end of storage period.

the pre-treatments. In the later harvested, mature tubers, however, the percentage of reducing sugars decreases during storage. Generally speaking, the potatoes stored for a brief period at 7° C. possess a higher reducing sugar content at the end of the permanent storage than those temporarily stored at 18° C. In contrast to total sugars the percentage of crude fibre increases during storage irrespective of the pre-treatment.

Table I

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Total sugars			Reducing sugars		
		At time of harvest	At the end of storage period		At time of harvest	At the end of storage period	
			Pre-storage at 7° C.	Pre-storage at 18° C.		Pre-storage at 7° C.	Pre-storage at 18° C.
27. xi. 34	Adolescent	1·03	0·39	0·41	0·208	0·272	0·232
30. xi. 34		1·02	0·36	0·39	0·201	0·271	0·220
3. xii. 34		1·03	0·35	0·37	0·198	0·272	0·219
6. xii. 34		1·04	0·30	0·35	0·147	0·271	0·215
9. xii. 34		0·97	0·28	0·33	0·132	0·221	0·199
12. xii. 34	Mature	0·88	0·27	0·32	0·122	0·217	0·197
15. xii. 34		0·68	0·26	0·31	0·112	0·208	0·188
18. xii. 34		0·57	0·23	0·30	0·109	0·197	0·176
21. xii. 34		0·49	0·21	0·27	0·107	0·096	0·075
24. xii. 34		0·31	0·18	0·25	0·103	0·078	0·060
27. xii. 34		0·28	0·18	0·23	0·101	0·064	0·051
30. xii. 34		0·27	0·17	0·20	0·097	0·059	0·049
2. i. 35		0·26	0·16	0·19	0·087	0·089	0·057
5. i. 35	Ripe	0·29	0·27	0·26	0·096	0·102	0·081
8. i. 35		0·28	0·23	0·25	0·098	0·106	0·089
11. i. 35		0·28	0·23	0·23	0·088	0·110	0·087
14. i. 35		0·27	0·22	0·20	0·049	0·049	0·036
17. i. 35		0·25	0·21	0·22	0·048	0·048	0·036

Table II

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Sucrose			Starch		
		At time of harvest	At the end of storage period		At time of harvest	At the end of storage period	
			Pre-storage at 7° C.	Pre-storage at 18° C.		Pre-storage at 7° C.	Pre-storage at 18° C.
27. xi. 34	Adolescent	0·789	0·138	0·139	11·01	11·56	11·89
30. xi. 34		0·744	0·130	0·137	11·09	11·84	12·21
3. xii. 34		0·837	0·129	0·134	11·75	11·91	12·85
6. xii. 34		0·903	0·127	0·133	11·79	11·92	12·86
9. xii. 34		0·837	0·126	0·129	12·23	12·96	12·98
12. xii. 34	Mature	0·673	0·120	0·128	13·75	13·11	13·37
15. xii. 34		0·567	0·117	0·127	14·01	13·87	13·92
18. xii. 34		0·327	0·117	0·126	14·07	13·99	14·03
21. xii. 34		0·295	0·115	0·125	14·61	14·01	14·46
24. xii. 34		0·197	0·114	0·124	15·02	14·91	14·92
27. xii. 34		0·184	0·113	0·122	14·91	14·77	14·87
30. xii. 34		0·139	0·121	0·131	14·79	13·92	14·15
2. i. 35		0·147	0·172	0·136	14·29	13·88	14·06
5. i. 35	Ripe	0·189	0·149	0·156	14·01	13·92	14·02
8. i. 35		0·187	0·139	0·156	13·93	14·81	14·93
11. i. 35		0·187	0·130	0·147	13·44	14·69	15·01
14. i. 35		0·145	0·129	0·144	13·45	14·92	15·02
17. i. 35		0·144	0·127	0·141	13·49	14·92	15·01

Table III

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Moisture: At time of harvest	Moisture: At the end of storage period: Pre-storage at 7° C.	Moisture: At the end of storage period: Pre-storage at 18° C.	Total nitrogen: At time of harvest	Total nitrogen: At the end of storage period: Pre-storage at 7° C.	Total nitrogen: At the end of storage period: Pre-storage at 18° C.
27. xi. 34	Adolescent	83·67	80·21	81·31	0·29	0·38	0·34
30. xi. 34		83·29	80·07	81·57	0·29	0·38	0·36
3. xii. 34		83·13	79·87	81·69	0·30	0·39	0·37
6. xii. 34		82·69	79·76	81·81	0·31	0·37	0·33
9. xii. 34		82·22	79·23	80·75	0·31	0·36	0·34
12. xii. 34	Mature	80·97	79·06	80·73	0·30	0·30	0·30
15. xii. 34		80·77	78·92	80·10	0·30	0·30	0·32
18. xii. 34		80·73	78·91	79·26	0·37	0·35	0·34
21. xii. 34		79·97	78·92	78·99	0·38	0·36	0·35
24. xii. 34		79·38	78·21	78·32	0·39	0·37	0·34
27. xii. 34		79·37	78·82	78·89	0·38	0·38	0·36
30. xii. 34		80·29	78·61	78·87	0·40	0·39	0·38
2. i. 35		80·23	78·42	79·62	0·40	0·39	0·39
5. i. 35	Ripe	80·73	79·12	79·67	0·42	0·47	0·43
8. i. 35		81·04	78·81	79·69	0·43	0·48	0·43
11. i. 35		81·07	78·87	79·73	0·38	0·43	0·40
14. i. 35		80·71	78·81	79·21	0·37	0·42	0·39
17. i. 35		80·93	78·80	79·07	0·38	0·42	0·38

Table IV

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Ash: At time of harvest	Ash: At the end of storage period: Pre-storage at 7° C.	Ash: At the end of storage period: Pre-storage at 18° C.	Crude fibre: At time of harvest	Crude fibre: At the end of storage period: Pre-storage at 7° C.	Crude fibre: At the end of storage period: Pre-storage at 18° C.
27. xi. 34	Adolescent	0·93	1·22	1·20	0·35	0·48	0·42
30. xi. 34		0·92	1·21	1·23	0·34	0·46	0·42
3. xii. 34		0·93	1·22	1·19	0·32	0·45	0·41
6. xii. 34		0·91	1·11	1·11	0·31	0·44	0·40
9. xii. 34		0·91	1·08	1·09	0·32	0·41	0·39
12. xii. 34	Mature	0·92	1·07	1·08	0·32	0·39	0·38
15. xii. 34		0·92	1·08	1·07	0·32	0·38	0·36
18. xii. 34		0·97	1·09	1·06	0·31	0·38	0·35
21. xii. 34		0·99	1·07	1·08	0·31	0·38	0·32
24. xii. 34		1·01	1·06	1·07	0·31	0·38	0·31
27. xii. 34		1·01	1·22	1·07	0·33	0·39	0·30
30. xii. 34		1·01	1·21	1·08	0·31	0·40	0·31
2. i. 35		1·02	1·17	1·09	0·32	0·38	0·31
5. i. 35	Ripe	1·02	1·16	1·16	0·35	0·36	0·30
8. i. 35		1·02	1·15	1·17	0·34	0·35	0·31
11. i. 35		1·03	1·13	1·17	0·32	0·35	0·31
14. i. 35		1·03	1·12	1·21	0·31	0·33	0·31
17. i. 35		1·03	1·19	1·22	0·33	0·31	0·31

Table V

Percentage values of losses due to respiration and transpiration in 10-lb. lots of tubers during the storage period

Date of digging	Developmental stage	Pre-storage at 7° C.		Pre-storage at 18° C.	
		Shrinkage by water loss	Shrinkage by respiration	Shrinkage by water loss	Shrinkage by respiration
27. xi. 34	Adolescent	14·94	1·01	11·95	0·98
30. xi. 34		13·30	0·97	11·23	0·88
3. xii. 34		12·14	0·92	10·22	0·87
6. xii. 34		12·00	0·93	9·53	0·78
9. xii. 34		11·29	0·96	8·60	0·67
12. xii. 34	Maturity	10·52	0·87	7·68	0·66
15. xii. 34		7·91	0·77	7·44	0·63
18. xii. 34		6·63	0·63	6·50	0·52
21. xii. 34		6·32	0·61	6·14	0·49
24. xii. 34		6·19	0·54	5·60	0·41
27. xii. 34		5·61	0·51	4·80	0·39
30. xii. 34		5·53	0·49	4·58	0·39
2. i. 35		5·53	0·44	4·08	0·35
5. i. 35	Ripe	4·56	0·39	3·63	0·34
8. i. 35		4·39	0·44	3·54	0·34
11. i. 35		4·20	0·43	3·35	0·37
14. i. 35		3·79	0·42	2·78	0·36
17. i. 35		3·54	0·41	2·32	0·27

Table VI

Physiological loss in weight of tubers at different depths in the bin

Date of digging	Developmental stage	Pre-storage at 7° C.			Pre-storage at 18° C.		
		Top layer	Middle layer	Bottom layer	Top layer	Middle layer	Bottom layer
27. xi. 34	Adolescent	10·29	15·95	19·52	8·68	12·93	16·97
30. xi. 34		10·01	14·27	18·53	8·12	12·11	16·12
3. xii. 34		9·79	13·06	16·33	7·29	11·09	15·08
6. xii. 34		9·29	12·93	16·57	6·99	10·31	14·37
9. xii. 34		8·11	12·25	16·12	6·23	9·29	12·28
12. xii. 34	Mature	7·92	11·39	15·39	5·95	8·34	11·37
15. xii. 34		5·65	8·68	11·69	5·29	8·07	11·08
18. xii. 34		4·27	7·26	10·27	5·09	7·02	9·08
21. xii. 34		3·91	6·39	9·96	4·98	6·63	8·79
24. xii. 34		3·46	6·73	9·62	4·91	6·01	8·09
27. xii. 34		3·32	6·12	9·13	4·82	5·19	6·79
30. xii. 34		3·17	6·02	9·01	3·92	4·97	5·98
2. i. 35		3·09	5·97	7·99	3·88	4·43	5·46
5. i. 35	Ripe	2·98	4·95	6·97	3·21	3·97	4·97
8. i. 35		2·97	4·83	6·84	2·99	3·88	4·91
11. i. 35		2·82	4·63	6·63	2·88	3·72	4·73
14. i. 35		2·76	4·21	6·21	2·83	3·14	4·71
17. i. 35		2·55	3·95	5·93	2·01	2·39	3·91

Of potatoes of all developmental stages the greatest loss in the sucrose content occurs in the earlier harvested, adolescent tubers. At the end of the storage period the sucrose content in all the lots was practically the same irrespective of the developmental stage of the tuber and the

pre-storage temperature. Nevertheless, a greater loss of sucrose was observed in the tubers pre-stored at 7° C. than in the lots placed at 18° C. for a brief duration previous to permanent storage. During the time when the tubers are maturing on the vines a slight increase in the sucrose content is noticed. It is significant to note that potatoes belonging to this lot continued to be distinguished by a higher sucrose content up to the end of the storage period. In contrast to the data obtained for sucrose, the quantity of starch increases in the adolescent tubers during storage under both the pre-treatments. At any rate, a distinct increase in the starch content is discernible in ripe potatoes at the end of the storage period. This is true of both the pre-treatments, though a greater starch accumulation took place in the lot temporarily stored at 18° C.

Tables III and IV show that the moisture content of potatoes decreases slightly during permanent storage irrespective of the pre-treatment. Greater loss of water occurred in the tubers pre-stored at 7° C. as compared to those maintained at 18° C. previous to permanent storage. The percentage of total nitrogen increases during storage in adolescent as well as ripe tubers under both the pre-treatments. In general, the potatoes pre-stored at 7° C. for a brief duration possess a higher total nitrogen content at the end of the storage period than the lots temporarily placed at the higher temperature. Moreover, a gradual increase in the ash content occurs with the advancement of maturity of the tubers. In the adolescent and mature potatoes this process continues in the storage (irrespective of the pre-treatment), so that, by the end of the storage period, the percentage ash in all the lots is practically the same and is equal to that found in the ripe tubers immediately after digging.

It is evident from Table V that the loss in weight of potatoes due to respiration is very small in comparison with that caused by evaporation of water. In the adolescent tubers the loss in weight during storage is high, and the value for total loss decreases with increasing maturity of the tubers. Tubers stored for 10–12 days at 18° C. lost considerably less weight during subsequent storage than did those stored for the same length of time at 7° C. previous to permanent storage.

The data concerning the effect of depth of piling potatoes on the loss in weight of tubers at different depths are presented in Table VI. Potatoes were placed in bins 4 ft. long and 3 ft. wide and were piled to a depth of $3\frac{1}{2}$ ft. in three layers, each layer 14 in. deep and separated from the other by $\frac{1}{2}$ in. mesh wire netting. The data show that comparatively less shrinkage occurs in the top layer than in the middle and the bottom ones.

While the work was in progress it was found desirable to study the effects of pre-storage temperature on the rapidity and the extent of suberization and wound-periderm formation. The material for histological examinations was selected from mature tubers which were divided into

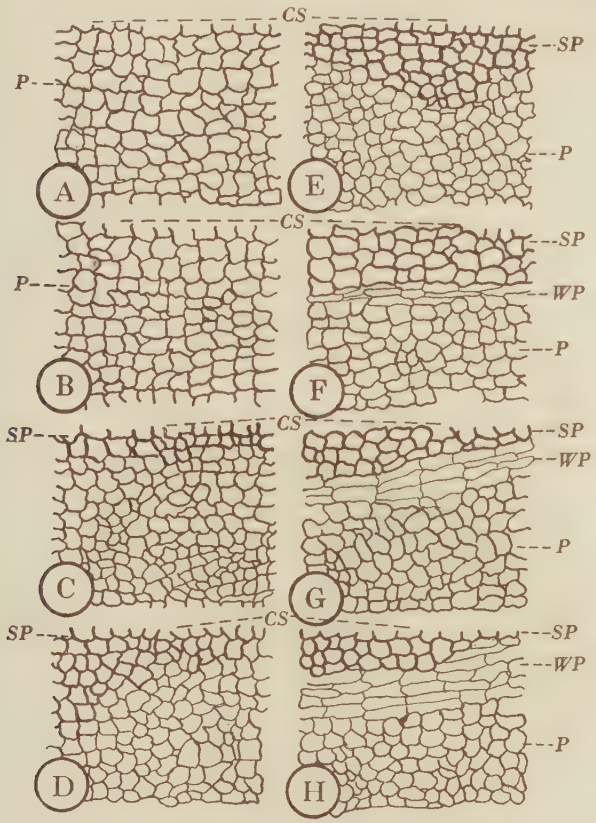

Fig. 2. Camera lucida drawings of sections of periderm of potatoes placed at 7°C. and and 18°C. for 12 days. *CS*, cut surface; *SP*, suberized parenchyma; *WP*, wound periderm and *P*, parenchyma. ×50.

two lots, one of which was placed in storage at an average temperature of 7° C. and the other at 18° C. for 12 days. Each day during the pre-storage period several wounded potatoes were selected from each of the storage temperatures and freehand sections were cut and examined microscopically for the appearance of suberization and periderm formation in the wounded areas. Camera lucida drawings of the suberized and

periderm layers of tubers in storage at the two temperatures are shown in Fig. 2. Suberization first made its appearance on the ninth day in tubers stored at 7° C., whereas appreciable suberization was observed in the lot stored at 18° C. as early as the third day. Wound periderm was

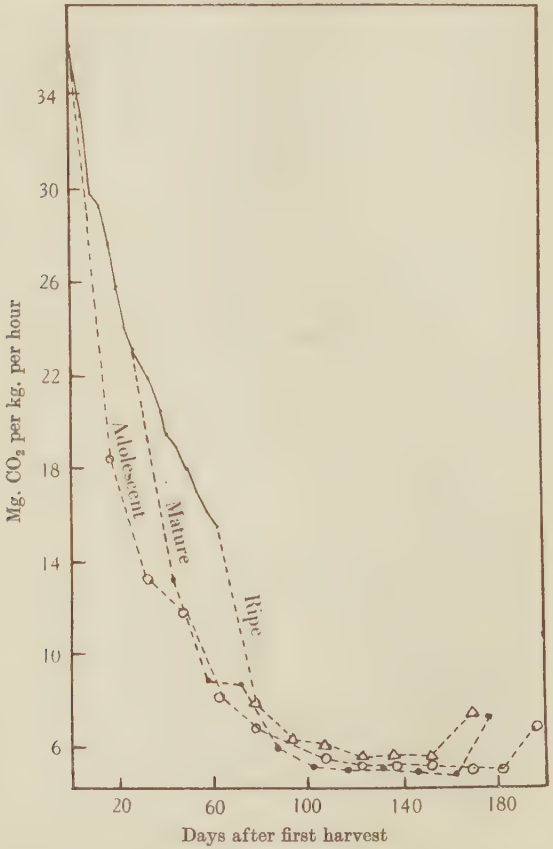

Fig. 3. Composite diagram showing the respiration intensity of potatoes during adolescence, maturity and ripening (continuous curve) and also the respiration rates of tubers of the same developmental stages during storage (discontinuous curves).

first noted on the sixth day of storage at 18° C., while the tubers stored at 7° C. had no periderm layer even at the end of 12 days of storage. Fig. 3 is a composite diagram constructed from data obtained during 1934–5 as well as 1935–6. The bold continuous curve represents the rate of respiration of developing tubers during the growing season, and the

discontinuous curves represent the respiration rates of adolescent, mature and ripe tubers dug at intervals of 21 days and immediately placed in storage at $13 \pm 0{\cdot}8°$ C. The decrease in the respiration rate is much more rapid during storage than with those potatoes that remain in the soil for the same length of time. Another interesting feature brought out by Fig. 3 is that, starting from the day of harvest, the initiation of sprouting is hastened more and more with the increase of the time during which the tubers are allowed to remain in the soil. In other words, if potatoes of all developmental stages be stored on the same day the immature ones will keep in storage the longest. To take a concrete example, counting from the first day of harvest the adolescent tubers begin to sprout 20–40 days later than the mature and ripe ones. This finding, together with our observation that mature potatoes possess at the end of the storage period practically the same percentage composition as the ripe ones, indicates the superiority of the former over the latter for seed purposes.

Appleman *et al.* (1928) demonstrated that during the storage of potatoes the loss in weight due to respiration is considerably less than that due to transpiration. Smith (1934) has brought forward evidence which shows that a large amount of water may be lost from the tubers before the healing of the wounds inflicted during harvesting is completed. Artschwager (1927) came to the conclusion that a temperature of 69·8° F. and a relative humidity of 95% are the lowest optimum conditions for rapid wound-periderm formation. Weiss *et al.* (1928) have shown that, at temperatures below 50° F., even at favourable humidity, the tubers are not able to form a protective layer which will exclude the more virulent rot organisms.

A greater loss in weight of adolescent tubers during storage in comparison with mature and ripe ones appears to be due to the fact that, in the former, the periderm layer is incompletely formed and the tubers are, in consequence, more susceptible to injuries during harvesting. Evidently a greater loss of moisture and carbon dioxide will occur from heavily wounded immature tubers. Smith (1929) showed that the more immature the tubers are when harvested the greater is their rate of respiration. This evidence likewise helps to explain the large amount of shrinkage from the adolescent potatoes.

The lessening of shrinkage after a pre-storage at 18° C. as contrasted with a temporary storage at 7° C. may be explained on the assumption that the process of wound-periderm formation is hastened at the higher temperature thus curtailing the loss in weight during subsequent storage.

Obviously the lower temperature retards the process of suberization and periderm formation in the wounded areas, and therefore the period of rapid evaporation and high rate of respiration is prolonged. This results in a greater loss in weight of tubers pre-stored at the lower temperature. It is desirable to hasten the process of wound-periderm formation in order to reduce the loss in weight by increased evaporation and respiration from the wounded areas or from the uninjured parts on which periderm formation is not yet complete.

Summary

1. Tubers stored for 10–12 days at 18° C. lost considerably less weight during subsequent storage than those pre-stored at 7° C. previous to permanent storage. This emphasizes the importance of pre-storing potatoes for a brief duration at a higher temperature preparatory to permanent cold storage.

2. In the adolescent tubers the loss in weight during storage is high and decreases with increasing maturity of the tubers, the value for the total loss being about the same in mature and ripe tubers.

3. Although the magnitude of shrinkage during storage of mature and ripe potatoes is practically the same, the former are superior to the latter in that they keep longer in storage without sprouting.

4. During storage the loss in weight of potatoes due to respiration is very small in comparison with that caused by evaporation of water.

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STUDIES IN POTATO STORAGE

III. RESPIRATION OF POTATO TUBERS DURING STORAGE

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(With 3 Text-figures)

In a recent communication (Singh & Mathur, 1937) from this laboratory it was shown that during its development the potato tuber passes through three more or less well-defined stages designated as adolescence, maturity and ripening. In this connexion an important question that suggested itself was whether potatoes belonging to the three above-mentioned stages are distinguishable in their respiratory behaviour during the storage period. If specific differences occur in the respiratory behaviour of potatoes of different developmental stages such studies may prove fruitful in solving certain problems of gas-storage of tubers, for success in storing tubers in various gaseous mixtures depends upon the correctness with which the rate of respiration during the subsequent period of storage can be predicted. These considerations led to experiments, the results of some of which are briefly described.

Methods

The tubers (variety Farrukhabad) were dug at intervals of 3 days from the crop belonging to the season 1935-6 and each lot, on its arrival in the laboratory, was immediately stored at a fairly constant temperature of 12–13° C. Altogether eighteen lots were stored, the first lot being dug on 1 December 1935 when the plants were in full blossom, and the last on 21 January 1936 when the vines were beginning to dry. During the period of storage, respiration intensity, internal gaseous concentrations, composition of the atmosphere surrounding the tubers as well as values of R.Q. were determined regularly at 15-day periods. Data were also obtained with regard to the permeability of the periderm of the potato, the general method of Smith (1929) being employed.

Respiration measurements were made by the method of continuous aspiration; the carbon dioxide evolved was trapped in caustic soda and titrated against standard acid. The respiratory quotient was measured

by enclosing the material in airtight respiration chambers, and the gaseous samples removed were analysed from time to time by means of an adaptation of Haldane's gas-analysis apparatus. By employing the

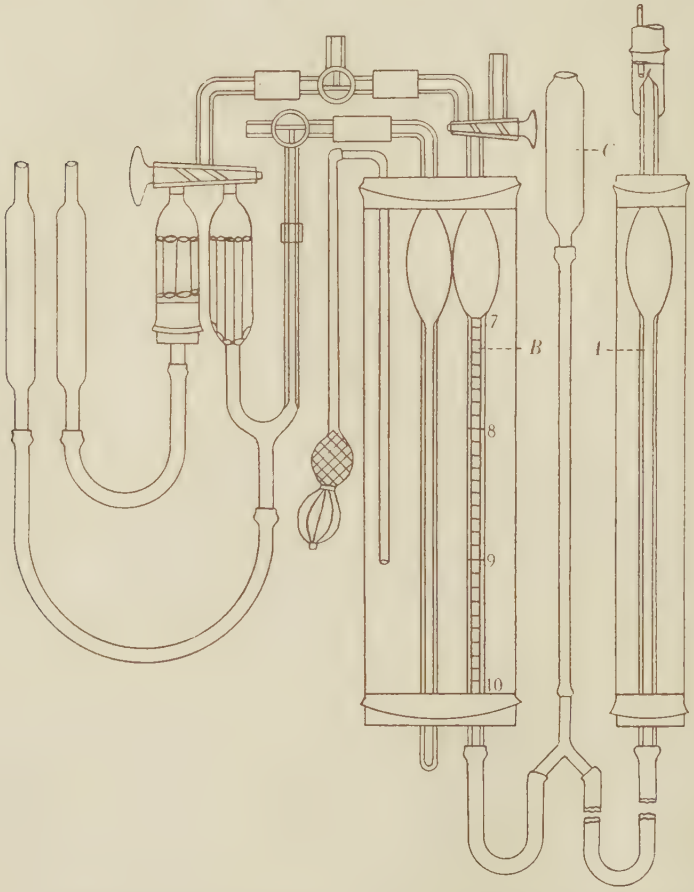

Fig. 1. Haldane gas-analysis apparatus adapted for making mechanical reductions to N.T.P. For explanation see text.

principle[1] of Lunge's gas-volumeter (Lunge & Keane, 1924; Morris, 1933) the apparatus has been improved in that the values are mechanically

[1] The principle of the apparatus is to enclose a known volume of air at such a pressure that it takes up exactly the volume which it would occupy at 0° C. and 760 mm. pressure. If the same pressure is then applied to another volume of gas this would also take up the volume which it would occupy at N.T.P.

reduced to N.T.P. without paying any attention to the readings of the thermometer and the barometer. In the modified apparatus (Fig. 1) which has been in use for about two years in this laboratory all samples of gas are measured as dry gas at N.T.P. For this purpose the "reduction" tube (*A*) is connected with the graduated pipette (*B*) and the mercury levelling bulb (*C*). Readings are taken by slightly loosening the clamp of the tube (*A*) so that it slides up or down and raising or lowering the levelling bulb till the mercury occupies the same level both in the reduction tube (*A*) and in the measuring pipette (*B*). The gas in (*B*) now occupies the volume which it would occupy when dry at N.T.P. Further details of manipulation of this apparatus have already been given by Singh & Mathur (1936*a*, *b*.).

For the determination of the composition of the interior gas, plugs of tissue from stem to eye end, 1 in. diameter, were removed from five tubers of each lot. One plug at a time was cut and pressed out of the cork borer under mercury into the gas-extraction apparatus described by Smith (1929). For determining the composition of the air surrounding the tubers, the gas samples were withdrawn with a modification of the sampler described by Singh & Mathur (1935) and analysed by means of Singh & Mathur's (1936*a*) adaptation of the Haldane gas-analysis apparatus.

Table I

Respiration intensity[1] *of potatoes of different stages of development during storage*

Date	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1. xii. 35	37·2	18·5	13·3	11·9	8·2	6·9	6·4	5·4	5·3	5·1	4·9	5·1	4·8	6·7
4. xii. 35	35·3	18·1	12·8	11·2	8·2	6·1	6·2	5·3	4·9	4·7	4·8	4·9	6·8	—
7. xii. 35	33·1	17·2	12·1	11·2	7·1	5·9	5·2	5·1	5·0	4·8	5·1	5·0	5·0	—
10. xii. 35	29·9	17·1	11·8	10·9	6·4	6·0	5·1	5·0	4·9	4·8	4·9	4·9	5·1	—
13. xii. 35	29·3	16·5	11·8	10·8	6·3	5·9	5·0	4·9	5·1	4·8	5·1	5·2	4·9	—
16. xii. 35	27·7	15·3	11·3	10·1	6·1	5·3	4·9	4·8	5·0	5·1	6·9	6·7	—	—
19. xii. 35	25·8	14·9	9·9	9·2	5·5	5·1	4·8	4·9	4·9	5·1	7·1	6·7	—	—
22. xii. 35	24·1	13·8	9·7	8·1	5·7	5·2	4·9	4·9	4·8	4·9	7·0	8·1	—	—
25. xii. 35	23·2	13·3	8·9	8·7	5·8	5·0	4·9	5·0	4·8	4·8	7·1	7·0	—	—
28. xii. 35	22·4	12·7	8·7	8·2	5·2	5·4	5·2	5·1	5·1	6·7	7·4	—	—	—
31. xii. 35	21·9	12·2	8·7	8·1	5·8	4·9	4·8	5·1	5·1	5·0	7·7	—	—	—
3. i. 36	20·5	10·0	7·9	7·3	5·7	4·8	4·9	5·0	4·9	4·9	6·8	—	—	—
6. i. 36	19·5	9·8	7·8	7·2	6·1	4·7	4·8	4·9	4·9	5·1	5·0	—	—	—
9. i. 36	18·8	9·3	7·8	7·1	6·2	4·8	4·9	5·0	4·9	5·1	—	—	—	—
12. i. 36	18·1	9·2	7·5	7·4	6·3	4·7	5·0	4·8	5·2	6·6	—	—	—	—
15. i. 36	17·0	9·1	7·1	7·0	6·2	5·0	5·0	4·9	4·9	6·7	—	—	—	—
18. i. 36	16·1	8·2	7·0	6·9	5·0	4·7	4·8	4·7	7·9	—	—	—	—	—
21. i. 36	15·5	7·9	6·2	6·1	5·5	5·6	5·4	7·3	—	—	—	—	—	—

[1] Milligrams of carbon dioxide per kg. per hour.

Results

The respiration data are presented in Table I, and a few typical records shown graphically in Fig. 2. Immediately after digging the

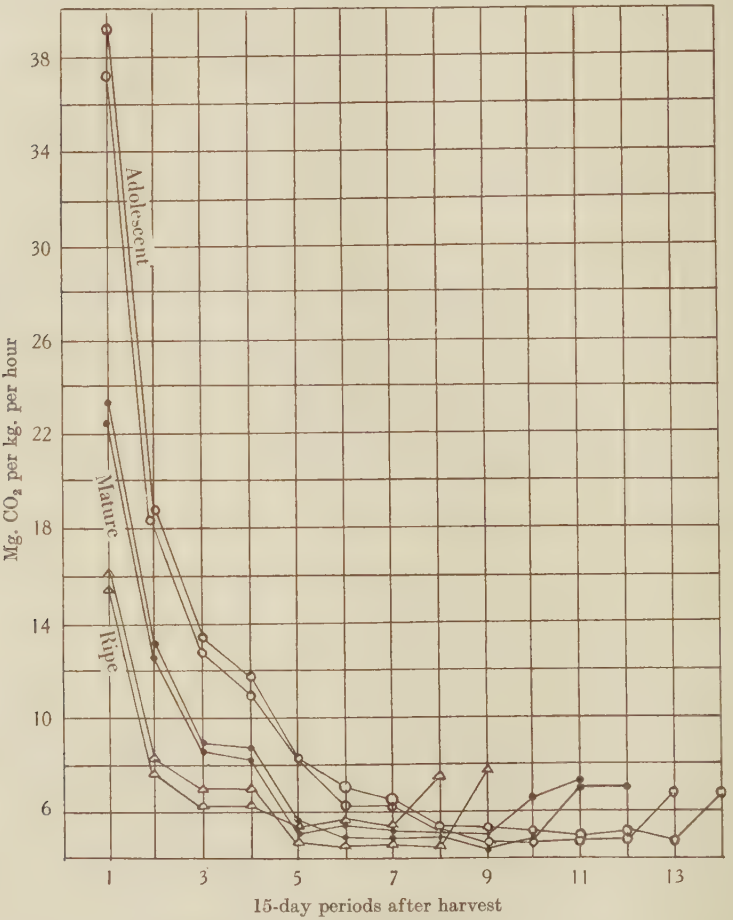

Fig. 2. Respiration rates of adolescent, mature and ripe potatoes during the storage period.

adolescent tubers exhibit a very high respiration intensity which decreases, at first rapidly and then gradually, during storage. Sooner or later the respiration intensity drops down to a level phase, the rate of respiration usually increasing again with the sprouting of the tubers.

In mature and ripe tubers the fall in respiration rate during the first fortnight is less steep and the condition characteristic of dormancy is entered into sooner. After being placed in storage the ripe potatoes are the earliest to terminate their period of dormancy.

The data regarding internal gaseous concentrations are shown graphically in Figs. 3 A and B. In the adolescent tubers the increase in the internal concentration of carbon dioxide is slow and gradually attains a more or less level phase, the concentration dropping rapidly with the commencement of sprouting. In ripe potatoes, on the other hand, the increase in the carbon dioxide concentration is rapid and is followed after the attainment of a peak value by an equally steep fall. The curves for oxygen concentration (Fig. 3 B) form more or less a mirror image of those for carbon dioxide. An increase in carbon dioxide concentration is accompanied by a corresponding decrease in that of oxygen, the value $CO_2 + O_2$ remaining practically constant.

Data concerning the composition of the atmosphere surrounding the tubers during storage are given in Figs. 3 C and D. In general, there is an accumulation of carbon dioxide in the surrounding air with increasing periods in storage. The concentration of carbon dioxide is the highest in the atmosphere surrounding the adolescent tubers, probably due to a high rate of respiration of the immature potatoes. The percentage of oxygen in the atmosphere surrounding the tubers progressively decreases with an increase in the length of the storage period.

Table II records the composition of samples of air withdrawn from top, middle and bottom layers. Mature potatoes were placed in bins 4 ft. long and 3 ft. in diameter and were piled to a depth of $3\frac{1}{2}$ ft. in three layers, each layer 14 in. deep and separated from the adjoining one by $\frac{1}{2}$ in. mesh wire netting. The data show a slightly higher percentage of

Table II

Effect of depth of piling potatoes on the composition of the atmosphere surrounding the tubers

Layer	At time of harvest	15-day periods after harvest 1	2	3	4	5	6	7	8	9	10	11	12	13
							Carbon dioxide							
Top	0·05	0·06	0·07	0·09	0·09	0·11	0·11	0·13	0·13	0·14	0·14	0·15	—	—
Middle	0·05	0·07	0·08	0·09	0·11	0·12	0·12	0·13	0·14	0·17	0·19	0·21	—	—
Bottom	0·05	0·09	0·11	0·12	0·14	0·21	0·22	0·25	0·27	0·29	0·31	0·31	—	—
							Oxygen							
Top	20·8	20·7	20·5	20·2	19·8	19·7	19·6	19·6	19·5	19·4	19·2	19·1	—	—
Middle	20·8	20·0	19·9	19·8	19·8	19·7	19·6	19·5	19·4	19·1	18·8	18·7	—	—
Bottom	20·8	19·9	19·7	19·6	19·4	19·2	18·9	18·7	18·6	18·6	18·6	18·6	—	—

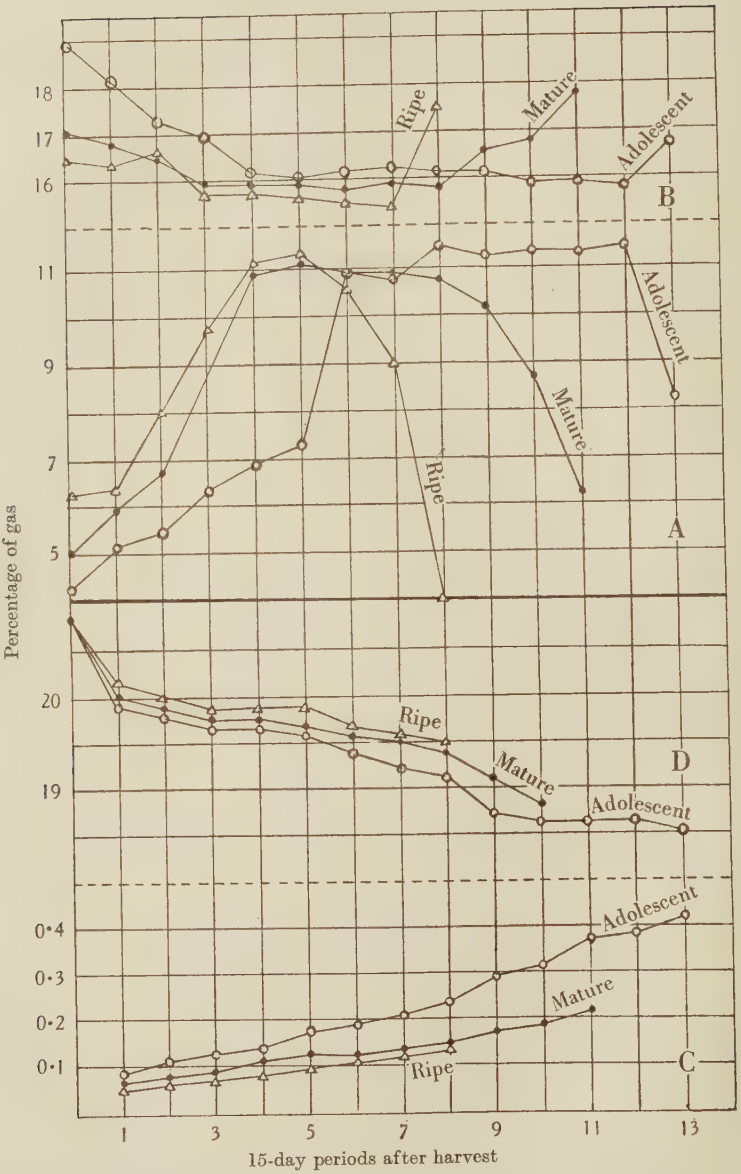

Fig. 3. Composition of the internal and external atmospheres of tubers during storage. A, internal carbon dioxide; B, internal oxygen; C, external carbon dioxide and D, external oxygen.

carbon dioxide in the bottom layer than in the middle and top ones. This higher percentage in the bottom layer may have been due to any or all of the following factors: greater respiratory activity, inability of gases to escape, and accumulation from layers above due to the density of the gas.

The values of R.Q. during storage are presented in Table III. In adolescent tubers the R.Q. starts with a value of about unity, drops down to 0·90 during the period of dormancy and rises again to 1·01 at the time of sprouting. A similar course of events applies to mature and ripe potatoes. A significant point in this connexion is the negative correlation between R.Q. and the internal carbon dioxide concentration during the periods of dormancy and sprouting (Fig. 3 A and Table III), higher values of R.Q. being invariably associated with lower carbon dioxide concentrations. It appears probable that with the onset of dormancy more and more carbon dixoide accumulates within the tissues of the tuber on account of a decrease in the superficial evolution of the gas.

Table III

Values of R.Q. *of potatoes of different developmental stages during storage*

Developmental stage	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Adolescent	0·98	1·00	0·99	0·99	0·98	0·98	0·97	0·91	0·90	0·90	0·91	0·90	0·91	1·01
Mature	0·99	1·02	1·00	1·00	0·91	0·90	0·91	0·91	0·92	0·92	0·99	1·03	—	—
Ripe	0·99	0·99	0·98	0·95	0·94	0·90	0·92	0·92	0·99	—	—	—	—	—

Data concerning the permeability of the periderm of the potato to gas during storage (Table IV) indicate that the permeability of the superficial tissues decreases considerably during the dormancy of the tubers, a slight increase being discernible during sprouting. This explains the accumulation of carbon dioxide and the depletion of oxygen in the tubers during dormancy.

Table IV

Permeability of the periderm of the potato to gas during storage

Developmental stage	At time of harvest	15-day periods after harvest											
		1	2	3	4	5	6	7	8	9	10	11	12	13
		Gas extracted from eight potatoes, ml.[1]												
Mature	5·6	5·0	5·0	4·8	4·3	4·1	3·6	3·1	3·1	3·2	4·5	4·6	—	—

[1] Average of ten determinations.

Discussion

In the case of storage organs, the problem of the relation between the rate of respiration on the one hand, and the internal and external concentrations of carbon dioxide on the other, is of considerable physiological significance. Its theoretical importance lies in the fact that the rate of respiration—the amount of carbon dioxide evolved superficially per unit weight—is influenced considerably by the composition of the atmosphere within the plant organs, which is in equilibrium with the amount of gas dissolved in the tissue sap as well as by the concentration of carbon dioxide in the surrounding atmosphere, which forms the immediate environment of the tubers. Since the permeability to gases of the periderm of the potato varies during storage of tubers it might be inferred that the superficial evolution of carbon dioxide will not bear a fixed ratio to its production during metabolism, as a result of which both the composition of the internal atmosphere of tubers as well as the rate of respiration will fluctuate within certain limits. The rate of carbon dioxide evolution under various circumstances will be conditioned, therefore, among other factors by (1) the concentration of carbon dioxide inside the tuber, (2) the composition of the atmosphere surrounding the tubers, and (3) the permeability of periderm to diffusion of gases.

In potato storage studies, therefore, considerable attention should be given to the development of the periderm and the various factors that influence its permeability to gases.

Summary

1. Adolescent, mature and ripe potatoes continue to be distinguished by their respiratory behaviour throughout the period of storage.

2. When potatoes are placed in storage there is a progressive increase in the concentration of internal carbon dioxide until the termination of the period of dormancy, the percentage of this gas falling rapidly with the commencement of sprouting.

3. Data concerning the composition of the atmosphere surrounding the tubers show that, in general, there is an accumulation of carbon dioxide in the surrounding air with increasing periods in storage.

4. There is a negative correlation between R.Q. and the percentage internal carbon dioxide during the stages of dormancy and sprouting.

5. Records concerning the composition of samples of air withdrawn from top, middle and bottom layers of potatoes show a slightly higher

percentage of carbon dioxide in the bottom layer than in the middle and top ones.

6. Data concerning the permeability of the periderm of the potato to gas during storage indicate that the permeability of the superficial tissues decreases considerably during the dormancy of tubers.

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FUNGI CAUSING ROTS OF APPLE FRUITS IN STORAGE IN NORTHERN IRELAND

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CONTENTS

I. Introduction

The investigation of the fungi responsible for the decay of apple fruits under ordinary storage conditions was undertaken in order to obtain some knowledge of the parasites responsible for storage losses in Northern Ireland, and to bring the position of the Province into line with that of other apple-growing countries where, in practically every case, information on this subject is available.

Up to the time of the commencement of the work little attempt had been made to study the storage rots of Irish-grown apples, so that few published records as to the fungi responsible for such losses are in existence. Lafferty & Pethybridge (1922) reported the occurrence of *Phytophthora Syringae* Klebahn as causing a rot of Irish apples. Muskett *et al.* (1931) reported the occurrence of *Phytophthora cactorum* (Leb. & Cohn) Schroet. and *Tricothecium roseum* Link on apples in Northern Ireland, and of *Penicillium expansum* Thom (Muskett *et al.* 1934), but pathogenicity was not tested. During the course of the present investigation, *Corticium centrifugum* (Lev.) Bres. was found to be the cause of a rot observed on a few apples grown and stored in Co. Armagh. The occurrence of this fungus was reported shortly after its identification (Colhoun & Muskett, 1935).

II. Experimental methods

During the years 1934 and 1935 visits were made at frequent intervals to a number of apple stores at orchards in the counties of Armagh, Tyrone and Antrim, this area comprising the most important apple-growing districts in Northern Ireland. From these stores typical samples

of the various rots occurring were selected, and isolations made from all rots present on these specimens. When pure cultures of the fungi isolated had been obtained they were tested for pathogenicity by carrying out artificial inoculations on apples of the variety Bramley's Seedling, the method of inoculation adopted being that described by Granger & Horne (1924). The inoculated apples were placed in moist chambers and held at laboratory temperatures. Where a rot was produced in the inoculated apples, a reisolation of the parasite was made. Organisms which did not produce rots in apples artificially inoculated at the beginning of the storage season were tested for pathogenicity at intervals until the end of the season.

III. THE FUNGI CONCERNED

The total number of species and strains isolated numbered over forty, and of these the following have been proved pathogenic to Bramley's Seedling apples:

Phytophthora Syringae Kleb. (strains 1, 2 and 3).
Mucor racemosus Fres.
M. piriformis Fischer.
Penicillium expansum Thom.
Botrytis cinerea Pers. (strains 1, 2 and 3).
Corticium centrifugum (Lev.) Bres.
Phoma mali Schulz & Sacc. (strains 1 and 2).
Phomopsis mali Roberts.
Cytosporella mali Brun.
Gloeosporium fructigenum Berk.
Colletotrichum gloeosporioides Penz.
Trichothecium roseum Link.
Fusarium lateritium Nees var. *fructigenum* (Fr.) Wr.
Fusarium avenaceum (Fr.) Sacc.
Sporotrichum sp.
Verticillium sp.

In addition to these species a number of other organisms have been proved pathogenic but have remained sterile on the apple fruit and on all media employed, and consequently have not been identified.

Practically all the organisms isolated were found producing rots on apples of the variety Bramley's Seedling which is the main variety grown in Northern Ireland. In the following account of the fungi and the rots they produce the variety of apple from which the organisms were originally isolated was Bramley's Seedling unless otherwise stated.

Phytophthora Syringae Kleb.

There appear to be only two species of *Phytophthora* responsible for disease of apple fruits in nature, these being *P. Syringae* Klebahn and *P. cactorum* (Leb. & Cohn) Schroet., of which the latter appears to have the wider distribution as regards its occurrence on apples, having been recorded in England (Wormald, 1919), Northern Ireland (Muskett *et al.* 1931), Switzerland (Osterwalder, 1906) and the United States (Clinton, 1920; Haskell & Wood, 1922; Rose & Lindegreen, 1925; Whetzel & Rosenbaum, 1916; U.S. Dep. Agric. 1918). *P. Syringae* has been recorded as being prevalent in the south-west of England (Ogilvie, 1932), and in the Irish Free State (Lafferty & Pethybridge, 1922).

During the present investigation three distinct strains of *P. Syringae* were isolated and studied, these being obtained from apples in the early part of the storage season when considerable damage is often caused. All three strains are capable of producing rots of healthy apples. The rots produced are mid-brown in colour, fairly firm to the touch in the early stages of the disease, but become softer later. The diseased internal tissue is light brown in colour with the vascular strands showing up as darker brown threads. No mycelium was observed on the surface of the apple.

The strains of *P. Syringae* studied produced sporangia when grown on 2% oat agar or on 2% malt extract agar, although the production of sporangia was more frequent in some cases. The production of sexual organs on either of these media was not observed, but when small pieces of diseased tissue from inoculated apples were incubated in sterile water for a number of days, oogonia and antheridia, which were predominantly paragynous, were produced.

The technique described by Leonian (1934) was employed in assigning the strains to the species *P. Syringae*. When plates of Leonian's medium were inoculated with any of the three strains and incubated at 27° C. no growth resulted. Dishes of sterile pea broth were inoculated with hyphae of each strain and incubated at 20° C. for 3 days. The colonies formed were then thoroughly washed in sterile distilled water, and transferred to Petri dishes which contained enough sterile distilled water to cover their bottoms. After 6 days' incubation at 20° C. it was found that all three strains produced sporangia freely, but sexual organs did not develop. The sporangia when mature were rounded or slightly flattened at the apex and without a definite papilla, thus resembling those of the organism described by Lafferty & Pethybridge (1922) as being *P. Syringae*.

Many workers have reported that infection of apples with *Phytophthora* takes place from the soil so that windfalls are chiefly liable to be attacked. A considerable number of apples were collected in October 1935 on a damp day, from the ground under trees in a grass orchard, and allowed to dry in the laboratory. They were then carefully examined and any showing signs of infection with disease were discarded. The remainder were stored in a cool room for 18 days and at the end of this period at least 25% of them showed infection with *Phytophthora*. A number of cases have been reported in Northern Ireland where apples on pulling had been allowed to lie on the ground in the orchard for a few days before storing, and after two or three weeks in store showed signs of *Phytophthora* rot.

Lafferty & Pethybridge (1922), in reporting the occurrence of *P. Syringae* in Ireland, did not consider that it was likely to become a serious menace to Irish fruit growers, but the present study shows that the disease may, under certain conditions, be of some importance in Northern Ireland.

During this investigation an organism isolated from pears, and which is believed to be *P. cactorum*, was studied. This organism is also pathogenic to apple fruits.

Mucor racemosus Fres. and *M. piriformis* Fischer

The species of *Mucor* occurring on apples in Europe may be referred to *M. racemosus* Fres., *M. mucedo* Linn., and *M. piriformis* Fischer. All three species have recently been reported as causing rots in Bohemia (Baudys, 1931). In America *M. piriformis* has been recorded (Heald & Ruehle, 1931), but it is stated to be an infrequent cause of decay.

Two species of *Mucor* were isolated during the study, and these considered together rank among the four most important diseases of stored apples occurring in Northern Ireland. They proved to be *M. piriformis* and *M. racemosus*, the latter being the more common. Both are more prevalent during the early part of the storage season. The rots produced by the two species are indistinguishable, the lesions are light brown in colour, soft and watery, and the rot progresses very rapidly. The diseased internal tissue is light brown in colour and very soft. In well-rotted fruits sporangiophores appear at breaks in the skin or emerge through lenticels. When well-rotted apples are kept in an airtight chamber for some days a characteristic alcoholic odour is noticed.

Penicillium expansum Thom.

A considerable number of species of *Penicillium* are responsible for loss in stored apples, but of these *P. expansum* is by far the commonest. During the present survey *P. expansum* was the only species of the genus isolated. It appears to be widespread in the province, being the most serious source of damage to stored fruit. The rot produced is light brown in colour, soft and spreads very rapidly. The diseased internal tissue is very watery and pale in colour with the vascular strands showing up as darker brown threads. Spores are produced in coremia on the surface of the apple quite early in the development of the rot and in great abundance. In the early stages of its development the sporulating growth is white in colour, but as the spores mature it becomes blue-green. Well-rotted apples often exhibit a musty odour. *P. expansum* usually gains entrance to the fruit through wounds but is also capable of penetrating lenticels (Horne & Eweis, 1934).

Botrytis cinerea Pers.

B. cinerea is the most common species of this genus occurring on the apple fruit, and it has been reported from most apple-growing countries. A number of distinct strains were isolated during this work, and while all these strains are capable of causing rots they differ in some respects as regards cultural characteristics, chiefly in their capacity to produce conidia and sclerotia.

The rots produced by the various strains are indistinguishable, being light brown in colour and firm to the touch but becoming softer later. A few weeks after inoculations are made whitish tufts of fungal growth appear on the surface of the apples. Conidiophores bearing conidia have been frequently observed on apples at points where the skin was broken.

Corticium centrifugum (Lev.) Bres.

This species has been recorded in Europe on dead twigs, etc. (Burt, 1926), but has not been observed to cause disease of home grown apples in Britain until isolated during the present survey (Colhoun & Muskett, 1935). In 1930 the disease was recorded in England on apples imported from Canada (Rome, 1931). It has been studied in America, where it is widespread, by Eustace (1903), Butler (1927, 1930), and Heald & Ruehle (1931). The earlier workers considered the disease to be due to *Hypochnus* sp., but Butler (1930) assigned the causal organism to the genus *Corticium*.

The lesions resulting from natural infection may or may not centre

around scab spots, but when a scab spot occurs in the centre of a lesion there is a marked resemblance to the eye of a fish, the black patch in the centre caused by the scab fungus (*Venturia inaequalis*) being surrounded by tan-coloured tissue with a ring of dark brown around the outside. Lesions often coalesce to form a large diseased patch. The diseased area is usually quite firm and slightly sunken. When diseased apples are kept in a moist chamber the fine white mycelium of the fungus forms a cobweb-like mat over the surface of the fruit. The production of spores on the apple fruit was not observed. The diseased internal tissue is light brown in colour, dry and stringy, giving the whole a somewhat spongy consistency.

The fungus grows readily on most of the common media, and on malt agar, which was the medium chiefly used in the work, basidiospores are produced in abundance, and clamp connexions develop on the mycelium. These characters, together with a sweet, penetrating, aromatic odour emitted by cultures, assist in the identification of the fungus.

In Northern Ireland the disease has only been found in one orchard, and in that case only a few apples were affected. The disease is essentially a late storage rot. Butler (1930) has suggested that infection of the fruit takes place in the orchard, the fungus establishing itself in tissues of the mature fruit which are dead or at a low ebb of life, such as stems, calyces or lenticels. The disease then develops later in storage. Both Butler (1930) and Eustace (1903) considered that the fungus could not penetrate sound epidermis.

Phoma mali Schulz & Sacc.

Apart from the record of Massee (1915) who, when working on blister disease of fruit trees in England, decided that *Coniothecium chomatosporum* Corda, *Phoma mali* and *Diaporthe ambigua* Nits. are different stages of one organism, which causes a blister disease of young shoots and also attacks the fruit, this species has been proved by Lewis (1909) to be capable of causing a rot of apples in America.

In the present study two strains of *P. mali*[1] have been found causing rots of apples. The rot produced on artificial inoculation by strain 1 is light brown in colour and firm, but becomes softer as the disease progresses. The rot spreads rapidly in ripe apples, and about 4–5 weeks from the time of inoculation, pycnidia appear beneath the skin of the apple. A few weeks later the pycnidia burst through the epidermis and

[1] Both strains were kindly identified by Dr Joh. Westerdijk, Centraalbureau voor Schimmelcultures, Baarn.

masses of small oval spores are exuded from each. The spores which measure $7{\cdot}1 \times 3\mu$ are slightly larger than those reported by Lewis (1909). Strain 2 produces a rot which is dark brown in colour and very firm to the touch, but within 10 days from the time of inoculation, a large number of pycnidia appear just under the skin of the diseased area and finally the whole surface becomes black. About a month after inoculation the pycnidia burst through the cuticle and the spores emerge. These spores measure $7{\cdot}1 \times 2{\cdot}9\mu$.

The behaviour of the strains has been studied on a number of different media. Strain 1 has never produced spores in culture on any of the media employed, although sterile black bodies have developed usually on 2% oat agar or on Crabill's medium. Strain 1 produced slight blackening of the medium when grown on malt extract or potato mush agar, but no blackening has been observed on oat or prune agar or on Crabill's medium. Strain 2 produced abundant unilocular pycnidia and spores on most media, where it also caused very distinct blackening of the medium.

P. mali appears to be of considerable importance in bringing about loss of stored apples in Northern Ireland, especially towards the close of the storage season. From observations made in the stores it appears that the fungus gains entrance chiefly through the stalk end of the fruit.

Phomopsis mali Roberts

This organism, which is regarded as the pycnidial stage of *Diaporthe perniciosa* Marchal, has been recorded on apple fruits in England (Kidd & Beaumont, 1924) and America (Roberts, 1912). Kidd & Beaumont (1924) state that the fungus causes much loss late in the storage season. This has been confirmed in Northern Ireland where, in a number of stores, *Phomopsis mali* was the dominant species causing rots from Christmas onwards.

The rots produced are mid-brown in colour, fairly firm to the touch and spread rapidly. In well-developed rots small pycnidia appear just below the skin and are densely crowded all over the fruit. Eventually these pycnidia, which are unilocular, burst through the skin and liberate small masses of spores, both A and B spores being produced.

In old cultures a few large black stromata are produced. The pycnidia are unilocular and produce masses of A and B spores.

Cytosporella mali Brun[1]

This species was recorded in 1924 for the first time on apple fruits by Kidd & Beaumont (1924) in England, where it was isolated on one occasion.

In Northern Ireland it was isolated on three occasions during the survey. The rots from which isolations were made were centred around injuries or the stalk end of the fruit. The rot produced by the fungus is dark brown in colour, becoming darker towards the centre of the diseased area. Pycnidia are produced abundantly in culture but their production on diseased fruit has not been observed.

Gloeosporium fructigenum Berk.[1]

This species appears to have a wide distribution and has been reported from most apple-growing countries. In England it was isolated on two occasions by Kidd & Beaumont (1924). In Northern Ireland the fungus does not appear to be of much importance in causing rots of apples, since it has only been isolated on one occasion from a few apples towards the end of the storage season. The rot produced is dark brown in colour and slightly soft.

Colletotrichum gloeosporioides Penz.

This fungus, which is known to be a common parasite of oranges, has been previously recorded in England on apples by Kidd & Beaumont (1924), who found it occurring on one occasion.

During the present work *C. gloeosporioides* was isolated on one occasion from a few apples of the variety Golden Spire, and it has been observed in another orchard on apples of the variety Bramley's Seedling. The rot produced on Bramley's is dark brown in colour and rather soft. As the rot spreads, the pinkish coloured acervuli appear in great abundance on the surface of the fruit.

In culture on malt agar the acervuli appear in abundance, but with frequent subculturing the power to produce spores decreases. The acervuli produced in culture and on the apple contain numerous dark coloured setae which are most easily seen in young pustules.

Trichothecium roseum Link

This is a widely distributed species and is stated on some occasions to cause considerable damage following scab. The organism was isolated in this survey on a large number of occasions from small spots centring

[1] Thanks are due to Dr Joh. Westerdijk for identification of this species.

around scab lesions. In the store a whitish fungal growth appears over the surface of the scab spot, and later the tissue attacked by the fungus becomes sunken, forming a narrow brown coloured band around the scab lesion.

Fusarium lateritium Nees var. *fructigenum*[1] (Fr.) Wr.

As far as stored apples in Northern Ireland are concerned *F. lateritium* var. *fructigenum* does not appear to cause much loss since it was isolated once only. The strain isolated was but feebly parasitic even towards the end of the season, although its powers of bringing about attack increase as the season advances. The rots produced are dark brown and slightly sunken, but fructifications of the fungus are not produced on infected fruits. This organism was also successfully isolated from buds of the variety Grenadier from Co. Armagh, where it is responsible for causing a bud rot.

Fusarium avenaceum (Fr.) Sacc.[1]

In recording the occurrence of this species on apples in England, Kidd & Beaumont (1924) state that it is not of great importance in storage. During this study *F. avenaceum* was first isolated in the autumn 1934 from a small shrivelled apple remaining on the tree. Later it was isolated on four occasions from fruit stored at different orchards, but in no case was the damage severe.

The rot produced is dark brown in colour, fairly soft, and slow in spreading. In cases where the disease is well advanced the diseased portion of the flesh becomes sunken, and a weft of hyphae covers a large portion of the surface of the diseased tissue. As far as could be ascertained from observations in the store infection usually takes place through the stalk end of the fruit.

Sporotrichum sp.

This organism was isolated on a few occasions from small dark brown lesions towards the end of the storage season. Inoculation experiments show that it is feebly parasitic and will attack Bramley's Seedling apples only when the storage season is well advanced. It has not yet been possible to assign the organism isolated to a definite species.

Verticillium sp.

This organism was isolated on one occasion during the work and was found to be feebly parasitic even at a very late period in the storage season. It has not yet been definitely identified.

[1] Cultures of both species of *Fusarium* were kindly identified by Dr Wollenweber.

IV. Discussion

During this investigation the variety Bramley's Seedling received most consideration, since this is the variety most largely grown on a commercial scale in Northern Ireland. Being a sour variety, and as, in this work, little attention was paid to fungi causing lenticel spotting at the close of the storage season, the number of organisms studied has been limited.

It was observed during the course of the work that a large proportion of the rots present on apples in storage in Northern Ireland centred around wounds or the stalk or calyx ends of the fruit. The rots centring around lenticels were comparatively few in number until the storage season was far advanced.

Horne (1931) has shown that the primary infection of apples contracting disease during storage can be traced to the orchard from which such apples were obtained, for it was found that the fungi present in the tissues of apples contracting disease were also present on the fruit before it was detached from the tree. Carter (1935), in her study of the fungi in air over orchards, produced evidence in support of the suggestion that diseases of apples in storage are due mainly to fungal infection from the atmosphere before the picking of the fruit. Further, Ogilvie (1935) has shown that many of the fungi capable of attacking apple fruits may also lead a parasitic or saprophytic existence on other parts of the apple tree, chiefly on the limbs.

It would therefore appear that much may be done to improve the keeping quality of the fruit by paying strict attention to hygienic conditions within the orchard. The exercise of greater care in general orchard management should do much to reduce the number of spores present in the atmosphere. The reduction of fruit losses due to fungal decay will also be achieved by carrying out regularly an efficient spraying programme for the control of diseases and pests; by the more careful handling of the fruit during and after picking; and by taking care not to leave the fruit lying on the ground in the orchard after picking.

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COMPLEX FUNGAL ROTTING OF PEA SEEDS

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(With Plates I and II)

CONTENTS

Introduction

Poor germination of peas of both garden and field varieties sometimes reaches such proportions as to be a limiting factor in pea growing. The causes of these "poor plants" in England have never been definitely determined. Several fungi are important in causing diseases of the stems or other parts of peas, and these may be placed in three groups distinguishable by the symptoms produced.

Group I

Fungi causing primarily spotting of the pods, and later of the cotyledons of the peas, which leads eventually to a poor stand of plants and a certain amount of distortion of the stems with more or less foot-rot.

Mycosphaerella pinodes Berk. & Blox. According to Ogilvie & Mulligan (1932) this fungus can cause severe foot-rot in England, in addition to blight of the pods and leaves as described by Jones (1927).

Ascochyta pisi Lib. Ogilvie & Mulligan (1932) found that this fungus, primarily a leaf-spotting and pod-spotting organism, causes little foot-rot, producing only brown sunken lesions on the underground part of the stem.

Ascochyta pinodella L. K. Jones. Jones (1927), who names this species, found it to cause foot-rot in addition to spotting of the pods and leaves.

Group II

Fungi causing primarily wilting, accompanied occasionally by slight foot or root-rot.

Fusarium orthoceras App. & Wr. var. *pisi* Linford. Apart from typical wilt symptoms, the plants infected by this fungus show a limited superficial browning of rootlets but are otherwise white and clean externally. Marked vascular discoloration occurs (Linford, 1928).

Fusarium oxysporum Schl. f. 8, Snyder. Causal organism of an important wilt, called "near-wilt" of peas in U.S.A. Snyder & Walker (1935) found that, after 8 weeks' growth in soil heavily inoculated with the fungus, many of the small roots of pea plants were bronzed and decayed, but the underground macroscopic symptoms were on the whole slight when compared with the severe wilting which occurred.

Fusarium viticola Thuem. on wilted pea, Sweden (Togashi, 1928–9).

Fusarium culmorum (W. G. Smith) on wilted pea, Sweden (*ibid.*).

Fusarium dimerum Penzig, on wilted pea, Sweden (*ibid.*).

Fusarium vasinfectum Atk. var. *pisi* van Hall, causing St John's wilt in Holland (*ibid.*).

Fusarium conglutinans Wr. on wilted pea in U.S.A. (*ibid.*).

Group III

Fungi causing primarily root and foot-rot.

Aphanomyces euteiches. According to Jones & Drechsler (1925) this fungus enters only the cortex of the roots and base of the stem where it softens and decays the tissue, exposing the vascular system to decay by other organisms. According to Jones & Linford (1925) in their survey of Wisconsin pea diseases "during the 1924 survey the root-rot of peas caused by *Aphanomyces* was found to be far more important than all the other parasitic diseases combined, causing losses amounting to approx. 8 % of the yield of the total acreage inspected".

Rhizoctonia solani. Miss Gilchrist (1926) illustrates this fungus causing severe foot-rot, the cotyledons being practically rotted away, and considerably more severely infected than with *Ascochyta pinodella.* Jones & Linford (1925) state that this fungus causes greatest injury when invading very young tissues, though it may attack any underground portion of the plant. They further state "It may enter germinating seeds,

killing the embryo or destroying the cotyledons, removing the food reserve of the developing seedlings. It may attack seedlings before emergence from the soil, injuring or completely destroying the growing points of roots and stem."

Fusarium solani (Mart.) var. *martii* (Appel & Wr. subspecies) Wr. f. 2, Snyder. This fungus, originally named by Jones (1923) *Fusarium martii* var. *pisi*, and renamed by Wollenweber and Snyder, has come to be recognized as one of the most important and widespread of the pea root-rotting fungi. Various strains of this fungus, and single spore strains in various conditions of culture, show wide variability (Snyder, 1934). Hence the original contention of Jones, that this fungus "must be distinguished on morphological characteristics which are constant", has had to be modified, and Snyder uses pathogenicity as a major characteristic in distinguishing this fungus from closely related forms and subspecies of *F. solani*.

This fungus causes a severe rotting of the stem and roots, discolouring them reddish brown to almost black. It may cause rotting of the cotyledons. It may, in addition, cause a wilt, as indicated by Jones (1923). According to Johanna Went (1934) it is a chief cause of St John's wilt in Holland. According to F. R. Jones it is the only species of *Fusarium* causing serious foot-rot in the U.S.A., and Ogilvie *et al.* (1934) hold the same views for England.

Other species of *Fusarium* which have been mentioned in the literature are the following:

F. arthrosporioides Sherb.
F. sporotrichioides Sherb.
F. anguioides Sherb. (Togashi, 1928–9).
F. avenaceum (Fr.) Sacc. (*ibid.*).
F. herbarum (Corda) Fr. (*ibid.*).
F. herbarum var. *gibberelloides* Wr. (*ibid.*).
F. martii Appel & Wr. (*ibid.*).
F. martii var. *minus* Sherb. (*ibid.*).
F. falcatum Appel & Wr. (*ibid.*).
F. redolans Wr. (*ibid.*).
F. blasticola Rostrup (*ibid.*).
F. scirpi Lamb. & Fautr. var. *acuminatum* (Ell. & Everh) (Wollenweber & Reinking, 1935).
F. equiseti (Corda) Sacc. var. *bullatum* (Sherb.) Wr. (*ibid.*).
F. merismoides Corda (*ibid.*).
F. equiseti (Corda) Sacc. (*ibid.*).

The descriptions given of the various diseases by the different authors rarely leave any doubt as to the group in which any of the causal organisms may be placed. Throughout the literature, however, stress is laid upon one of the symptoms, pod spotting (with subsequent lesions developed on the young peas), wilting or root-rotting. In spite of the importance of securing a good "stand" of this crop, the rotting of seed in the soil is frequently overlooked.

Substantial increases in stand resulting from dressing pea seed have been known for some time, and the writer had the opportunity to investigate cases where very spectacular improvements were obtained from dressing seed with certain experimental dressings. The experiments were conducted in five widely separated localities. Recently a paper has been published by Brett *et al.* (1937) in which similar experiments with mercurial seed dressings gave large increases.

Early experiences showed that it was no simple matter to isolate parasitic fungi from rotting seed; usually an abundance of moulds and bacteria was the reward of large numbers of isolation plates. As a supply of the original seed had been secured from each of the five centres, this served to supply the necessary information on diseases carried on the seed. Naturally, *Ascochyta* and *Mycosphaerella* were sought as the disease organisms. In addition, stems and roots of almost mature plants were secured from each centre; they showed an abundance of superficial rotting and served as an indication of root and stem parasites present. Finally, a number of stock cultures of widely separated fungi, mostly obtained from plants other than peas, completed the source of biological material.

Experimental methods

(*a*) *Isolation of cultures*

A simple method of surface sterilizing tissue was found very satisfactory in isolating *Ascochyta pisi*, many species of *Fusarium* and other fungi from stems, roots and seeds of peas. It is similar to that described by Davies (1935) for isolating *Ophiobolus graminis* from wheat stems. The tissue was soaked for 10–60 min. in distilled water, dipped for 2–3 min. in 1 % silver nitrate solution, washed in concentrated sodium chloride solution, and plated on potato-dextrose agar.

(*b*) *Media*

The media used for identification of fungi were those of Wollenweber *et al.* (1925). Ridgway's colour chart (1912) is used throughout for colour descriptions. In making certain spore measurements, assistance was given by Mr R. P. Libbey of Reading University.

(c) *Testing for pathogenicity*

The fungi were grown for 3 weeks in a sterilized mixture of 190 g. sand, 10 g. cornmeal and 80 c.c. water in flasks. This was mixed with three times the quantity (air-dry weight) of sterilized or unsterilized sandy loam. 100 g. were placed in a tumbler, ten pea seeds were sown, and the seeds were covered with more infested soil. The moisture was brought to 50 % saturation. The seedlings were grown at a convenient temperature. In one instance, boxes containing twenty-five seeds were used.

Fungi isolated

(a) *Fungi isolated from seed*

Table I indicates the types of fungi isolated from pea-seed samples taken from that used in the five field trials referred to above. In the table are given the variety of pea sown in each locality together with the average percentage increase resulting from the use of the five experimental dressings. These figures are a conservative indication of the benefit of seed dressings, inasmuch as they include one dressing distinctly inferior to the others in preventing rotting. Thirty seeds of each variety were sterilized with silver nitrate and plated on potato-dextrose agar. In separate columns are listed the number of peas showing complete freedom from contamination, the number showing species of either *Penicillium* or *Aspergillus*, and those showing other fungi, including an occasional isolate of a species of *Fusarium*.

Table I

Fungi isolated from seed used in field experiments

Variety	Locality	Increased field germination %	Peas free from contamination No. per 30 seeds	Peas showing *Penicillium* or *Aspergillus* only No. per 30 seeds	Peas showing other fungi No. per 30 seeds
Queen	Peterborough	15·8	29	0	1
Thomas Laxton	Rochester	58·6	16	10	4
Market Gardener	Rochford	50·9	2	22	6
Foremost	Evesham	3·2	22	7	1
Thomas Laxton	Rainham, Essex	16·7	22	1	7

None of the peas yielded parasitic fungi in appreciable quantities. The *Penicillium* and *Aspergillus* species were found to be non-parasitic, and among the "other fungi" neither *Ascochyta* nor *Mycosphaerella* was present. This was in spite of the fact that the method used for isolating has proved satisfactory for isolating *Ascochyta* from seeds, as will be seen later. It is evident that if the cause of rotting in the field was seed-borne

fungi, then they were very superficially borne and were destroyed by surface sterilization.

Seeds of four commercial varieties of peas well known to suffer from poor germination were then examined. Seeds of each variety were sown in unsterilized field soil in glazed earthenware pots, and others were sown in similar soil which had been sterilized in an autoclave at 20 lb. pressure for 2½ hr. After 14 days' growth at 50° F. the plants were lifted, and one cotyledon from each plant was surface sterilized with silver nitrate. The fungi isolated from the cotyledons of each variety are listed in Table II.

Table II

Isolation from cotyledons of seedlings of varieties Union Jack, Thomas Laxton, Early Bird, and Little Marvel

Variety	Soil	No. of plants examined	*Penicillium* only	*Fusarium* sect. *Elegans*	*Fusarium* sect. *Roseum*	*Fusarium culmorum*	*Botrytis cinerea*
Union Jack	Sterilized	22	18	4	0	0	0
	Unsterilized	13	0	11	1	1	0
Thomas Laxton	Sterilized	19	16	3	0	0	0
	Unsterilized	20	1	19	0	0	0
Early Bird	Sterilized	18	12	4	0	2	0
	Unsterilized	18	4	13	0	1	0
Little Marvel	Sterilized	13	10	2	0	0	1
	Unsterilized	22	10	11	0	1	0
Little Marvel	Sterilized	20	10	5	0	4	1
	Unsterilized	17	0	12	0	5	0

It will be noted that there was a strong tendency for *Fusarium* of section *Elegans* to occur on peas from unsterilized soil, while in the sterilized soil *Penicillium* was far more frequent. It seems not improbable that both fungi, or groups of fungi, were carried on the seed, one being favoured by sterilized soil and the other by unsterilized soil. It would also appear that *Fusarium culmorum* and *Botrytis cinerea* were carried on the seed. Several of the isolates tested later were found to be highly pathogenic, but they were not species of *Penicillium* or *Fusarium* section *Elegans*.

(*b*) *Fungi isolated from foot-rot material*

At the time when peas were picked from the five experimental centres referred to above, a large amount of foot-rot was present in all cases; in fact, it was difficult to find plants completely free from rotting. Plants were saved from each centre, no special effort being made to secure diseased material. At the same time pods showing spotting or rotting were collected from Evesham and Rochester. No pods were collected from the other centres.

The lower parts of the stem were dried and stored for several weeks, after which thirty pieces from each sample were surface sterilized and plated on potato-dextrose agar. A large number of the pieces proved to be sterile. A full list of the cultures isolated is given in Table III.

Table III

Fungi isolated from base of stems of plants (thirty pieces from each variety)

Variety	Locality	Pieces sterile	*Fusarium avenaceum*	*Fusarium culmorum*	*Fusarium solani Martii*	Other *Fusarium* spp.	*Botrytis cinerea*	Other fungi
Queen	Peterborough	13	13	0	0	1	0	3
Thomas Laxton	Rochester	19	0	0	2	3	0	6
Market Gardener	Rochford	10	5	0	0	2	1	12
Foremost	Evesham	17	0	0	1	10	0	2
Thomas Laxton	Rainham, Essex	14	0	0	0	8	1	7

(c) *Fungi isolated from diseased pods*

A few of the pods collected with the haulm from Evesham and Rochester showed a considerable amount of spotting typical of *Ascochyta*. They were dried and after a few weeks thirty seeds from the lightly infected pods were soaked, surface sterilized with silver nitrate and

Table IV

Fungi isolated from the seed in pods showing slight symptoms of disease, collected at Evesham and Rochester

Centre	No. of peas plated	Sterile peas	Peas yielding common moulds	Peas yielding *Ascochyta*	Peas yielding other fungi
Evesham	30	17	9	4	—
Rochester	30	2	24	—	4 (unidentified *Fusarium* spp.)

Table V

Fungi isolated from pods at Evesham and Rochester

Centre	Pod No.*	Fungus
Evesham (Plate I)	1	Unidentified fungus with sterile white aerial mycelium
	2	*Ascochyta pisi*
	3	*Ascochyta pisi*
	4	*Ascochyta pisi*
	5	*Ascochyta pisi* (?)
Rochester (Plate I)	6	Three species of *Fusarium*, unidentified
	7	Sterile fungus, resembling in colour and growth habit *Fusarium culmorum*
	8	Unidentified species of *Fusarium*
	9	*Botrytis cinerea*
	10	Unidentified fungus

* The pod numbers 1–10 refer to the position of the pods in Plate I.

plated on potato-dextrose agar. Five more heavily diseased pods from each centre, showing symptoms of *Aschochyta* and other fungi, were placed in a moist chamber for one week, when they showed abundant growth of fungi which were isolated. These ten pods were photographed before placing in the moisture chamber and are shown in Plate I. The fungi isolated from the seeds are listed in Table IV, and those from the pods in Table V.

It will be seen that there was a fairly heavy infection of *Ascochyta* at Evesham, while at Rochester this fungus was not secured, although the number of peas used was insufficient to say definitely that the fungus was not present. The results must not be interpreted as being an estimate of the relative abundance of *Ascochyta* at these centres, but serves to show that the method of surface sterilization used is quite suitable for obtaining this fungus from seed, and also to demonstrate that, under certain conditions, pea seed may harbour an appreciable fungal flora.

Pathogenicity of isolates

(*a*) *Isolates obtained from cotyledons of planted peas*

The cultures in this group are those referred to in Table II, obtained from cotyledons of seedlings of varieties Early Bird and Little Marvel. A representative of each fungus was tested in quadruplicate on 100 seedlings, variety Little Marvel, in unsterilized soil. Wooden boxes containing 25 seeds were used in a modification of Method II (*c*). The results obtained after 1 month's growth are given in Table VI.

Table VI

Results of inoculation of Little Marvel peas with isolates from diseased cotyledons of pea seedlings

Fungus	No. of seedlings from 100 seeds	Symptoms of rotting of plants on removal from soil after 1 month		
		Cotyledons	Roots	Stems
Fusarium culmorum from Early Bird	68	Severe	Moderate	Moderate
Fusarium sect. *Roseum* from Early Bird	5	Very severe	Severe	Severe
Fusarium sect. *Elegans* from Little Marvel	92	Slight	Trace	None
Penicillium species from Little Marvel	88	,,	,,	,,
Botrytis cinerea from Little Marvel	0	Very severe	—	—
Control	81	Slight	Trace	None

Fusarium culmorum, *Fusarium* of the section *Roseum*, and *Botrytis cinerea*, were obviously highly pathogenic and the last two named practically inhibited emergence of the seedlings. *Fusarium* section *Elegans* and *Penicillium* species were non-pathogenic.

(*b*) *Isolates obtained from foot-rot material*

Representatives of various fungi from the foot-rot material referred to in Table III were tested in duplicate for pathogenicity. The effects of these inoculations on the cotyledons, roots and stems are shown in Table VII.

Table VII

Effects of inoculating peas with foot-rot isolates

Source of isolate and fungus	Soil*	No. of seedlings from 20 seeds	Symptoms of rotting of plants on removal from soil after 21 days		
			Cotyledons	Roots	Stems
(1) Rochford:					
Fusarium avenaceum	+	0	Severe	—	—
	−	2	,,	Severe	Severe
Rhizopus species	+	20	None	None	None
	−	13	,,	,,	Trace
Fusarium species	+	18	,,	,,	None
	−	16	,,	,,	,,
Botrytis cinerea	+	0	Severe	—	—
	−	10	Moderate	Moderate	Moderate
Fusarium species	+	18	None	Slight	None
	−	14	,,	,,	Slight
(2) Rochester:					
Fusarium species	+	20	,,	,,	None
	−	8	,,	None	Slight
Dematiaceae	+	18	,,	,,	,,
	−	14	,,	Slight	,,
Fusarium solani var. *Martii* (?)	+	18	,,	,,	None
	−	10	,,	None	,,
(3) Rainham:					
Fusarium species	+	20	,,	,,	,,
	−	18	,,	,,	Slight
,,	+	20	,,	Slight	,,
	−	12	,,	,,	,,
Sterile mycelium	+	20	,,	None	None
	−	13	,,	,,	Slight
Fusarium species	+	19	,,	Slight	None
	−	11	,,	,,	,,
Dematiaceae	+	20	,,	None	,,
	−	16	,,	,,	,,
Fusarium species	+	20	,,	,,	,,
	−	5	,,	Slight	Slight
,,	+	20	,,	None	None
	−	17	,,	Slight	Slight
,,	+	20	,,	None	None
	−	20	,,	,,	,,
,,	+	20	,,	,,	,,
	−	12	,,	Slight	,,
,,	+	19	,,	None	,,
	−	17	,,	Slight	Slight
Macrosporium species	+	19	,,	None	None
	−	17	,,	Slight	Slight

* Sterilized (+), unsterilized (−).

Table VII (*cont.*)

Source of isolate and fungus	Soil	No. of seedlings from 20 seeds	Symptoms of rotting of plants on removal from soil after 21 days		
			Cotyledons	Roots	Stems
(4) Peterborough:					
Fusarium avenaceum	+	0	Severe	—	—
	−	0	,,	—	—
Fusarium species	+	18	None	None	None
	−	14	,,	Slight	,,
Fusarium avenaceum	+	0	Severe	—	—
	−	0	,,	—	—
,,	+	17	Moderate	Moderate	Moderate
	−	Not sown	—	—	—
(5) Evesham:					
Fusarium species	+	18	Moderate	Moderate	Moderate
	−	16	,,	,,	,,
Fusarium solani var.	+	19	None	Slight	None
Martii (?)	−	15	,,	None	,,
Fusarium species	+	20	,,	,,	,,
	−	15	,,	,,	,,
Control	+	20	,,	Slight	Slight
	−	17	,,	None	None
Control	+	20	,,	Slight	Slight
	−	14	,,	None	None
Control	+	20	,,	Slight	Slight
	−	18	,,	None	None
Control	+	20	,,	Slight	Slight
	−	16	,,	None	None

Of the twenty-seven isolates from rotted stems tested for pathogenicity, only six caused rotting of the cotyledons, stems and roots greater than that in the controls and these were all either *Fusarium avenaceum* or *Botrytis cinerea*. It will be noted that a fungus thought to be *Fusarium solani* var. *Martii* was not effective. A careful examination of this isolate showed that it could not be distinguished from *F. solani* var. *Martii* by morphological characteristics.

A culture of *Fusarium solani* var. *Martii* (?) was tested at a higher temperature (room temperature) in comparison with certain others of the above fungi in sterilized and unsterilized soil in duplicate tumblers of ten seeds, using 25% inoculum. Under these conditions a certain amount of rotting by this fungus occurred, together with marked blue coloration and rotting of the outer coating of the cotyledons, and considerable flecking of the stems. The reaction of this isolate was of a similar type but rather more severe than that produced by a culture of *F. solani* var. *Martii* supplied by courtesy of the Long Ashton Research Station through Mr Hickman. The fungus did not lower the percentage germination and could be said at most to be a weak parasite on pea seedlings.

(c) *Miscellaneous stock cultures*

Cultures of widely differing groups of fungi from a stock collection were tested in unsterilized soil, five replicates being used. Results obtained with the fungi in this group tested in unsterilized soil are shown in Table VIII.

Table VIII

Effects of inoculating peas with miscellaneous fungi

Fungus	No. of seedlings from 50 seeds	Symptoms of rotting of plants on removal from soil		
		Cotyledons	Roots	Stems
Rhizoctonia solani from rhizomes of *Agropyron repens*	46	Slight	Slight	Slight
Fusarium avenaceum from *Triticum*	48	Moderate	,,	Moderate
Fusarium culmorum from *Triticum*	34	,,	,,	Slight
Fusarium culmorum from *Dianthus*	33	,,	,,	,,
Fusarium species from *Vicia faba*	42	,,	Moderate	Moderate
Fusarium bulbigenum	46	None	Slight	Slight
Fusarium anguioides	48	,,	,,	,,
Fusarium species from *Tulipa*	48	,,	,,	,,
Botrytis cinerea from *Lactuca*	0	Severe	—	—
Ascochyta pisi from *Pisum*	46	Moderate	Slight	Slight
Botrytis cinerea from *Pisum*	0	Severe	—	—
Fusarium avenaceum from *Pisum*	30	,,	Severe	Severe
Cladosporium herbarum	48	Slight	Slight	Slight
Helminthosporium avenae from *Avena*	44	,,	None	None
Fusarium nivale from *Poa*	46	,,	,,	Slight
Penicillium digitatum	45	,,	Slight	,,
Fusarium culmorum from *Triticum*	35	Severe	Moderate	Moderate
Fusarium culmorum from *Callistephus*	39	,,	,,	,,
Fusarium Graminearum from *Triticum*	6	,,	Severe	Severe
Fusarium conglutinans from *Callistephus*	48	Slight	None	Slight
Fusarium Dianthi from *Dianthus*	46	,,	Slight	None
Fusarium species from *Tulipa*	47	Severe	Moderate	Moderate
Botrytis tulipae from *Tulipa*	47	Slight	Slight	Slight
Botrytis cinerea from *Rosa*	10	Severe	Severe	Severe
Helminthosporium sativum from *Triticum*	34	,,	,,	,,
Botrytis cinerea from *Lactuca*	0	,,	—	—
Helminthosporium teres from *Hordeum*	50	Slight	Slight	Slight
Botrytis cinerea	22	Severe	Severe	Severe
Fusarium coeruleum	49	Slight	Slight	Slight
Penicillium italicum	44	,,	None	None
Sclerotinia species (?) from *Lactuca*	0	Severe	—	—
Ophiobolus heterostrophus from *Oryza*	44	Slight	Severe	Severe
Control	47	Slight	Slight	Slight
Control	45	,,	None	,,

This group of fungi has again yielded interesting results. The fungi showing pathogenicity were:

Fusarium avenaceum from *Triticum.*
Fusarium culmorum from *Triticum.*
Fusarium culmorum from *Dianthus.*
Fusarium species from *Vicia faba.*
Botrytis cinerea from *Lactuca.*
Ascochyta pisi from *Pisum.*
Botrytis cinerea from *Pisum.*
Fusarium avenaceum from *Pisum.*
Fusarium culmorum from *Callistephus.*
Fusarium Graminearum from *Triticum.*
Fusarium species from *Tulipa.*
Botrytis cinerea from *Rosa.*
Helminthosporium sativum from *Triticum.*
Sclerotinia species(?) from *Lactuca.*
Ophiobolus heterostrophus from *Oryza.*

DISCUSSION

According to the literature a number of species of *Fusarium*, and in addition to some extent other fungi (*Mycosphaerella pinodes*, *Ascochyta pinodella*, *Aphanomyces euteiches* and *Rhizoctonia solani*), cause root-rot or foot-rot of peas in various parts of the world. It is also evident from the present work that many fungi are associated with the rotting of stems of mature plants, many of which cannot initiate rotting in young seedlings.

The results of these experiments show that in addition to those fungi recorded as occurring on pea seed in the literature, a number of fungi obtained from pea stems and from other sources cause severe rotting of the cotyledons. Of those tested, the following proved pathogenic:

Botrytis cinerea from *Lactuca*, *Rosa*, and elsewhere.
Fusarium avenaceum from *Triticum.*
Fusarium culmorum from *Triticum*, *Dianthus* and *Callistephus.*
Fusarium Graminearum from *Triticum.*
Fusarium species (unidentified) from *Vicia faba* and *Tulipa.*
Helminthosporium sativum from *Triticum.*
Sclerotinia species(?) from *Lactuca.*
Ophiobolus heterostrophus causes severe spotting without rotting of cotyledons.

It is clear from the present research that rotting of pea seeds in soil may be due to various fungi, and in nature presumably it will frequently be due to the combined effects of several. The greater pathogenicity of several fungi such as *Fusarium avenaceum*, *F. culmorum* and *Botrytis cinerea* in conjunction with their frequency of isolation and well-known wide distribution in soil suggest their importance. Stress need not be laid on the fact that isolates believed to be *Fusarium solani* var. *Martii* appeared only slightly pathogenic, as Snyder & Walker (1935) have stated that the only satisfactory means of distinguishing between forms of *F. solani* is by tests for parasitism on different hosts. That it can be an important parasite of peas has been fully established elsewhere.

The severe losses encountered in field experiments where seed dressings were not used can be put down to rotting of cotyledons, roots and stems caused by *Fusarium avenaceum* and other fungi probably including *F. culmorum* and *Botrytis cinerea*, but not seed-borne *Ascochyta pisi* and *Mycosphaerella pinodes*. The disease characteristic of that caused by the first three fungi has not yet been appreciated to the full, and it is possible that poor stands of peas (resulting in poor crops) are more frequently due to these fungi than to *Mycosphaerella* and *Ascochyta*, which can readily be detected on the seed and are, in fact, eliminated to a great extent by discarding poor seed.

The rotting of cotyledons of pea seed in the soil may be looked upon as a complex disease in which more than one fungus may be playing a part, but as far as the evidence goes, these fungi are distinctly parasitic in nature and the cotyledons can probably withstand an abundance of common moulds.

Summary

1. Poor stands of peas due to fungal rotting were observed in field experimental plots in widely separated pea-growing districts.

2. The rotting was not due to seed-borne *Ascochyta pisi* or *Mycosphaerella pinodes* which were apparently absent from the seed.

3. Isolations from surface sterilized cotyledons of several varieties of peas grown in sterilized and unsterilized soil showed an abundance of common moulds and several pathogenic fungi, namely: a *Fusarium* of the section *Roseum*, *Fusarium culmorum* and *Botrytis cinerea*.

4. Isolations from surface sterilized rotting stems from five experimental centres at harvest time indicated the presence of a great variety of fungi including a number of species of *Fusarium*. Many of these, including all the common moulds such as *Penicillium*, were non-pathogenic

to the cotyledons. *Fusarium avenaceum* was found to be highly pathogenic to the cotyledons; *F. solani* var. *Martii* showed some signs of causing rotting; *Botrytis cinerea* was highly pathogenic. Several non-pathogenic species of *Fusarium* were obtained.

5. Tests with numerous stock cultures show high pathogenicity of the following fungi to pea cotyledons:

Botrytis cinerea from *Lactuca*, *Rosa* and elsewhere.
Fusarium avenaceum from *Triticum*.
Fusarium culmorum from *Triticum*, *Dianthus* and *Callistephus*.
Fusarium species from *Vicia faba* and *Tulipa*.
Helminthosporium sativum from *Triticum*.
Ophiobolus heterostrophus from *Oryza*.
Sclerotinia species from *Lactuca*.
Fusarium Graminearum from *Triticum*.

6. It is suggested that loss of stand in pea crops due to rotting of the cotyledons by these and other fungi is probably more important than diseases caused by *Ascochyta* and *Mycosphaerella* and foot-rots in the late stage.

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EXPLANATION OF PLATES I AND II

PLATE I

Pods of peas collected at harvest time. Pods showing fungi as follows:

Figs. 1–5. Evesham collection

Fig. 1. Unidentified fungus with sterile white aerial mycelium.

Fig. 2. *Ascochyta pisi* and sterile fungus.

Fig. 3. *Ascochyta pisi*.

Fig. 4. *Ascochyta pisi*.

Fig. 5. *Ascochyta pisi*(?).

Figs. 6–10. Rochester collection

Fig. 6. Three unidentified species of *Fusarium*.

Fig. 7. Sterile fungus, resembling in colour and growth habit *Fusarium culmorum*.

Fig. 8. Unidentified species of *Fusarium*.

Fig. 9. *Botrytis cinerea*.

Fig. 10. Unidentified fungus.

PLATE II

Peas planted in soil heavily contaminated with *Fusarium avenaceum*

Fig. 1. Seed treated with a mercurial dressing.

Fig. 2. Untreated seed.

(*Received* 15 *July* 1937)

PADWICK.—Complex Fungal Rotting of Pea Seeds (pp. 100–114)

Fig. 2.

Fig. 1.

PADWICK.—Complex Fungal Rotting of Pea Seeds (pp. 100–114)

A DISEASE OF THE VIOLA CAUSED BY *RAMULARIA DEFLECTENS*

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(With Plate III and 2 Text-figures)

Viola plants of the variety Kate Blyth badly infected with *Ramularia* were received from Coventry in February 1934. The plants were set out in boxes and the fungus grown in culture.

Description on host

On the infected plants the fungus was confined to the leaves. Dark-coloured lesions, which as they grow older develop a white mycelium in the centre, start at the edge of the lamina and gradually spread inwards, often in the form of a semicircle. Often these lesions run together until the whole leaf, both on the upper and lower side, is covered with a dry white weft of hyphae. The conidiophores are branched, septate and toothed at the apex. The conidia vary from 6·9 to 24·1 μ in length and from 2·2 to 3·4 μ in breadth.

The fungus agrees with *R. deflectens* Bresadola (Lindau, 1907), the spore size being given as 18–40 × 5–7 μ. Although this is larger than the above measurements the description agrees otherwise, hence it has not been thought advisable to make a new species. In transverse sections of the leaves the black sclerotia-like bodies lie immediately under the epidermis. They measure from 40 to 70 μ in diameter and are found to vary in shape and size. The smaller ones are spherical, while the larger assume a flask shape with a definite papilla which protrudes through the ruptured epidermis. These flask-shaped bodies have hyphae protruding from the papilla and small spores, 3·4–5·4 × 2 2·2 μ, were found attached to their ends as Laibach (1921–2) observed in *R. knautiae* (Pl. III, fig. 1). These bodies are composed of three to four outer layers of cells with thick walls, while the interior is made up of smaller and thinner walled cells which appear lighter in colour. In a few cases small spores have been detected in the centre. These structures are evidently a form of pycnidia which do not, however, produce pycnospores in any great

number. Laibach in dealing with *R. knautiae* considers that these sclerotia are a form of perithecia which, for some reason, have been hindered in their formation and whose development has been led into another channel at an early stage. The fact that small spores have been found both within them and emerging from the papillae seems to suggest that they are really potential pycnidia. Perithecia have not been found on any of the plants examined.

Cultural methods

Cultures on plain agar were made from the conidia on the leaf and, after several days, monospore cultures were made from these on malt agar. The following media were found to be the most successful: malt agar, oat, pea, and bean agar.

Cultural characters

The optimum growth of the mycelium occurred on malt agar at 17° C. It was found by experiment that light does not affect the growth of this fungus.

(*a*) *Conidia.* In culture the conidia are cylindrical, laterally compressed and slightly bent. The mature spores are two-celled, while the youngest have only one cell. Lactic acid cotton blue is quickly absorbed by these conidia, but in the centre of each cell a clear vacuole is seen. When stained with Sudan III these vacuoles assume a bright red colour showing that they are oil drops. The conidia measure $5–11{\cdot}4 \times 2{\cdot}5–3{\cdot}5\,\mu$ with an average of $8{\cdot}4\,\mu$ (sixty measurements). Their attachment to the dentate septate conidiophore is terminal and as each new conidium is formed it causes the older conidia to be displaced laterally (Text-fig. 1). These conidia germinate within 24 hr. and either one cell or both cells may produce a germ tube (Text-fig. 2*a*). As growth proceeds cross septa are formed and branches from the germ tube are given off (Text-fig. 2*b*).

(*b*) *Pycnidia.* In multispore cultures after 3–4 months and in very old revived monospore cultures pycnidia were formed. The hyphae at different points in the culture took on a greenish tinge and when examined were seen to be greatly enlarged and clubbed together, while fusions between them had taken place. Finally this irregular green mass became black and formed the primordium of a pycnidium. The pycnidia possess a great range both in size and form, they vary from spherical to flask-shape and may measure from 100 to $200\,\mu$ in diameter. In section they show three outer layers of thick dark-walled cells, while the centre may be composed of light-coloured cells as in the young stage or may, when mature, be filled with spores which measure $4{\cdot}1–6{\cdot}6 \times 2–2{\cdot}5\,\mu$. The

Text-fig. 1. Conidiophore bearing conidia. Camera lucida. × 1700.

Text-fig. 2. *a*, germinating conidia. Camera lucida. × 2333. *b*, germinating conidium, later stage. Camera lucida. × 1750.

pycnospores are borne terminally, on extremely fine filaments which arise from a row of cells next to the innermost layer of thick-walled cells. The shape of the mature pycnidium was followed through in a series of microtome sections and was found to be flask-shaped with a definite ostiole (Pl. III, fig. 2). In culture the pycnidia were formed in zones as Klebahn (1918) noted in *R. hieracii*. A few pycnidia are usually formed at the point of inoculation, and these are surrounded by a pale zone in which no pycnidial formation takes place. Outside this a dark zone occurs in which pycnidia are present. As this zonation, which occurs throughout the culture, is present in different media and under different conditions of light and temperature, it would seem to be due to the constitution of the fungus and not to the environment (Pl. III, fig. 3). When microtome sections of the agar slope were examined it was noted that on the surface of the medium the pycnidia formed clusters of three to four, while within the medium they were borne singly. The following experiment proved that these pycnidia belong to the *Ramularia* and that their presence is not due to contamination. Pycnidia were washed in a solution of 1/1000 mercuric chloride for 3 min., removed to sterile water and washed thoroughly. Single pycnidia were then removed by means of a dry sterile needle, inoculated into tubes of malt agar and incubated at 17° C. After 5–6 days hyphae were observed growing from the circumference of the pycnidia. In two out of the six thus treated, pycnidia were reformed in the culture, while in the remaining four cultures pycnidia were absent, while conidia were found to be plentiful. The above experiment was repeated, but this time a 1% HCl solution for 5 min. was substituted, conidia when thus treated lost their power of germination, and so it is assumed that adhering conidia were killed as in the last experiment. Within 17 hr. a fine growth of hyphae was observed growing out from the pycnidia, while after 4 days all the cultures thus produced had formed pycnidia, and a marked zoning in the culture. The rapid germination of the pycnidia may be due to the stimulation caused by the action of the HCl upon the cells. In one or two cases pycnidia were placed upon a dry slide in a drop of sterile water and then burst by a sterile needle. The escaping pycnospores were then removed and put on to tubes of malt agar. After 3–4 days growth was observed. One week later the culture was found to contain typical conidia while pycnidia were subsequently formed.

In appearance they resemble very closely the so-called sclerotia of the leaf, and the following points of similarity were noted:

(1) The wall layers of both are identical in form.

(2) When seen in section the ripe sclerotia and pycnidia have a flask-shape with a definite opening.

(3) The pycnospores measure $4{\cdot}1–6{\cdot}6 \times 2–2{\cdot}5\,\mu$, while the spores borne on the hyphae of the papillae measure $3{\cdot}4–5{\cdot}4 \times 3–2{\cdot}2\,\mu$. Thus it is seen that there is a close approximation between the pycnidia formed in culture and the sclerotia in the tissue of the host.

(*c*) *Monospore cultures.* In monospore cultures after 3–4 days a white frill of hyphae is seen round the point of inoculation. This gradually increases in size until by the end of 7 days a small, circular clump of hyphae, which is floccose in appearance and projects from the substratum, is formed. The hyphae grow over the surface of the agar forming conidia freely, giving the culture a cream colour and forming a thickened rim at the growing edge. After 28–30 days the surface of the agar is entirely covered by the growth of the fungus, and conidial pustules are beginning to form from the substratum. These pustules are pale cream in colour and, in culture of 2 months, they are dotted over the entire surface of the slope. Monospore cultures on oat agar and on bean agar show the same appearance but the conidia are formed in greater abundance, the whole surface becoming covered by a deep cream coloured layer of spores. Similar results were obtained by growing the fungus on small sterilized pieces of Viola stem.

In cultural experiments the following facts have come to light:

(1) A monospore culture produced conidia but no pycnidia. After a resting period of 9 months this dried culture was revived by pouring fresh malt agar down the side of the tube. Pycnidia were now produced in abundance within 5 days.

(2) A multispore culture from the same culture as the above monospore produced pycnidia as well as conidia within 3 months.

(3) A subinoculation from this multispore culture continued to produce pycnidia, while a monospore culture from it formed conidia but no pycnidia.

It is obvious that pycnidia are formed both from monospore and from multispore cultures. Their formation is dependent on the lapse of some considerable period of time, but it seems that when pycnidial formation has once commenced it will continue even when subcultures are made. The period required for pycnidial formation appears to depend on the amount of the material used in making the culture. In the case of the multispore culture, where much material is transferred, the period is short, while on the other hand, in the case of the monospore culture

the period is considerably lengthened. Possibly this may be explained as the accumulation of some substance during the growth of the fungus. The whole question of the formation of the pycnidia seems to centre round the problem of nutrition, which is closely linked up with a time factor.

Perithecia. Perithecia were not obtained in culture. Various methods such as growing on special media, freezing and the addition of fungal extracts were tried but they all failed to induce perithecial formation. The perfect stage of the genus *Ramularia* does not appear to be found in culture. Laibach (1921–2), working on *R. knautiae*, and Klebahn (1918) on *R. hieracii*, both failed to find the perfect stage in culture, although it occurred in both these cases on the host plant and proved to be a species of *Mycosphaerella.*

Infection experiments

Infection experiments were carried out in August 1934. Viola plants of the varieties Aberdeen Blue and Mosley's Perfection were inoculated with a spore suspension from a monospore culture of *R. deflectens.* The plants were kept in a cold frame, but in no case were positive results obtained. By the beginning of October 1934 it was found that the original diseased plants of the variety Kate Blyth had become healthy and had lost all signs of the disease. Cuttings were made from these plants. In February the leaves of these plants were sterilized and then four were kept as controls while the other four were inoculated as above. After a week or two all four inoculated plants showed the presence of the fungus on the leaves (Pl. III, figs. 4, 5), while the four controls remained healthy. The spores measured $9{\cdot}5–35 \times 4{\cdot}7–5\mu$ $(22 \times 5\mu)$. Monospore cultures were made from these conidia and in culture the spores measured $8{\cdot}9–23 \times 4{\cdot}5–5\mu$.

It appears that there is a tendency for the conidia to be larger on the host than in culture. The failure of the infection experiments in August may have been due to the variety used, or to the fact that the plants are more resistant to disease at that time of the year.

Conclusion

Ramularia deflectens appears to be a weak parasite which only attacks the Viola in early spring. A cool moist atmosphere appears to favour the growth of the fungus. As perithecia have been found neither on the fresh material nor in culture, the correct systemic position of this

Fig. 1.

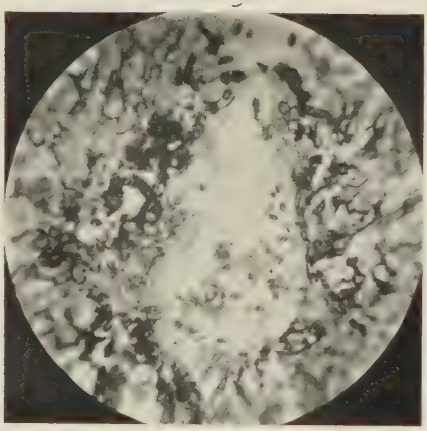

Fig. 2.

Fig. 4.

Fig. 3.

Fig. 5.

CAMPBELL.—A Disease of the Viola caused by *Ramularia deflectens* (pp. 115–121)

Ramularia cannot be decided on. The presence of pycnidia seems, however, to warrant its withdrawal from the Moniliales.

SUMMARY

1. Viola plants, of the variety Kate Blyth, which were badly infected with the fungus *Ramularia deflectens*, were received from Coventry in the early spring.

2. Sclerotium-like bodies were found on the leaves.

3. Pycnidia containing pycnospores were obtained in culture.

4. By comparison it is seen that these sclerotium-like bodies are really pycnidia which have not developed fully.

5. The formation of pycnidia in culture depends on nutrition.

6. Perithecia are found neither on the plant nor in culture.

7. In the early spring positive results from infection experiments are obtained.

The writer wishes to thank Dr Malcolm Wilson for his invaluable assistance and helpful criticism throughout the work.

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EXPLANATION OF PLATE III

Fig. 1. Transverse section. Leaf of viola showing pycnidium. Camera lucida. ×4000.
Fig. 2. Transverse section. Culture. Pycnidium showing ostiole. Photomicrograph. ×2000.
Fig. 3. Zoning in culture due to pycnidial formation. ×1¼.
Fig. 4. Infected plant showing presence of *R. deflectens*. ×½.
Fig. 5. Leaf of viola showing lesion caused by *R. deflectens*. ×6.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

VI. APHIS INFESTATION OF ISOLATED PLANTS

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The previous papers in this series have presented the results of studies on the aphis population of the potato crops in districts contrasting in the degree of spread of virus diseases (Davies, 1932, 1934, 1937; Davies & Whitehead, 1935) and also on the factors affecting migration of winged aphides (Davies, 1935–7; Davies & Whitehead, 1935). The present investigations were planned to ascertain the extent of the migrations of winged aphides, their ability to detect individual potato plants, the development of aphides on such units and the extent to which virus diseases were carried to these isolated plants.

Method

Centres were selected in districts contrasting in the usual rate of increase in virus infection among introduced healthy stocks of potatoes. Four centres were in the eastern part of North Wales where the spread of disease has been rapid, viz. Holywell, Dyserth and Hawarden in Flintshire, and Abergele in Denbighshire. Two were selected in South Caernarvonshire where there has been no appreciable increase in virus infection during the last nine years, viz. Madryn and Aberdaron. One other centre was at the College Farm near Bangor, which is intermediate in character but where the sources of possible infection were great since the experimental potatoes were planted in the same field as the infected plots of the virus museum.

At each centre, one hundred (or fifty) half-tubers of healthy stocks of Arran Comrade potatoes were planted separately in a root crop, with at least 6 yd. between the potato plants along the drill and 10 yd. across the drills of the mangold or sugar-beet crop. The corresponding half-tuber controls were planted at the College Farm in order to ascertain the health of the experimental plants; no virus symptoms appeared in these controls. Had it been practicable the experimental half-tubers would have been

grown on fallow land at the different centres in order to maintain complete isolation but, this being impossible, mangold and sugar beet were selected because at the time of appearance of the potato plants above ground, as well as at the time of migration of aphides, the potato units would be well separated and conspicuous as isolated plants. It was realized that *Myzus persicae*, the chief insect vector of virus diseases, is found occasionally on mangolds, but it is rarely present in considerable numbers before the foliage is large. Further, since the prevention of migration from one potato unit to another by crawling Apterae was being aimed at, the presence of another plant on which the migrating aphides might remain was no disadvantage. Cereal crops were not selected because they would have quickly smothered the potato foliage.

The half-tubers were planted at all centres in Flintshire on 10 May 1935, and in South Caernarvonshire 4 days later. The plants were first examined on 14 June when the majority were just through the soil and none was more than 4 in. high. The infestation of each species of aphis found was recorded from three leaves of each plant. When no aphides were found on the three leaves, which were always a random selection, the record was given as nil, but the whole plant was then examined for the presence of *M. persicae* and, if found, the number was recorded in brackets. These data were obtained at intervals during the season. In the autumn the progeny of each plant was separately lifted, boxed and removed to the College Farm, where it was stored until planted in the spring of 1936.

The aphis infestation of isolated plants at different centres

In an investigation of this nature it is important to study the history of each plant separately. The complete records from each centre are therefore presented, including the aphis population found at successive dates on each plant and the state of health of the progeny in the following year.

Centre I (Berthymaen, Holywell, Flintshire)

The aphis population on the potato crop at this centre has been observed since 1928 by the standard method of counting the population per 100 leaves, selected at random as the crop was traversed to and fro. The following figures give the "index-figure" of infestation for each year: 1928 (360, all spp.), 1929 (1910, all spp.), 1930 (550, *M. persicae*), 1931 (118, *M. persicae*), 1932 (1103, *M. persicae*), 1933 (848, *M. persicae*), 1934 (520, *M. persicae*), and 1935 (210, *M. persicae*). The rate of increase of

virus diseases at this centre was rapid, amounting to 33 % in 2 years (Whitehead *et al.* 1932.)

The isolated half-tubers at this centre were planted along the mangold rows, the nearest tubers being 40 yd. from the general farm crop of potatoes which occupied the same field. The hundred healthy, experimental half-tubers were planted on 10 May and seventy-five were above ground on 14 June but were not more than 4 in. high. The infestation record and subsequent health of the progeny of these plants are given in Table I.

Aphis infestation. The ability of winged aphides to detect and colonize isolated plants proved to be remarkable for, as can be seen in Table I, on 14 June, when the plants were very small, 61 % of those above ground were already infested with *M. persicae.* A fortnight later, on 28 June, when practically all plants had appeared above ground though none was more than 6 in. high, 98·9 % were infested with this species and at least 29 % carried *Macrosiphum gei* Koch. Further, there were nineteen *winged M. persicae* actually present on the forty-six infested plants (only three leaves on each plant being examined) on 14 June. It seems reasonable to assume therefore that the wingless forms found on the other plants had been produced by winged migrants which were either on leaves not examined or had again moved on to other plants. In fact, in most cases the nymphs, when present alone, were so small that it would have been impossible for them to have crawled any great distance across arable land. It follows therefore that, since the half-tubers were free from aphides when planted and no aphides were found on the seedling mangolds, the migrating winged aphides could detect and colonize isolated plants with ease, even in a season when the general aphis population was low in this district.

Spread of virus diseases by winged aphides. The health of the 100 half-tubers planted at this centre was confirmed by growing the corresponding halves at the College Farm, there being no symptoms of virus infection on these latter plants. The very considerable migration of winged aphides to the isolated plants at Berthymaen, shown in Table I, emphasized the importance of ascertaining the extent to which these migrants had infected the plants with virus diseases. The progeny of the plants was lifted in October 1935, removed and stored separately at the College Farm until spring, when four tubers of each unit were planted to ascertain their health. An examination of the last column in Table I indicates the remarkable amount of virus disease (94·6 %), particularly leaf-roll, which had been carried to the isolated plants in 1935; it may be added that in

Table I

Aphis population on isolated plants and evidence of virus transmission

Centre I (Berthymaen, Holywell, Flintshire). Potatoes isolated in mangold crop with farm potato crop 40 yd. distant in same field

Plant	Aphides present on three leaves on 14. vi. 35	28. vi. 35	26. vii. 35	Health of progeny 1936
1	3p	1g (p)	2p	Leaf-roll
2	1p*w*	1g, 10p	(p)	,,
3	–	1p	(p)	,,
4	1g, (p)	1p	(p)	Healthy
5	–	1p*w*	0	Leaf-roll
6	2p*w*	5p	1p	,,
7	–	9p	0	,,
8	1p*w*	12p	10p, 14g	,,
9	–	–	–	–
10	1p, 1p*w*	1p*w*, 3p, 1g	(p)	Leaf-roll
11	3p	(p)	(p, g)	,,
12	0	1p*w*, 3p	(p)	,,
13	(p)	(p)	(p)	,,
14	–	1p*w*, 5p	0	,,
15	1p*w*	1p	0	,,
16	1p*w*	1p	(p)	,,
17	1p*w*	4p, 1g	1p	,,
18	1p*w*	1p*w*, 2p	4p	,,
19	–	(p)	(p)	Healthy
20	–	1p	0	Leaf-roll
21	–	1p	0	,,
22	1p*w*	1p*w*, 6p, 1g	14p, 3g	,, + Interveinal mosaic
23	(p)	1p	(p)	,,
24	2p*w*	2p	(p)	,,
25	–	1p*w*, 2p	1g	Missing
26	0	1p, 1g	(p)	Leaf-roll
27	1p	1p, 2p*w*, 1g*w*	1p, 3g	,, + mosaic
28	(p)	2p*w*, 6p	(p)	,,
29	(p)	4p	(g)	,,
30	1p*w*	(p*w*)	(p)	Missing
31	0	1p, 1g	3p	Leaf-roll
32	1p	1p*w*, 1p	(p)	,,
33	1p	1p*w*, 5p	(p)	,,
34	0	8p, 2g	(p)	,,
35	(p)	(p)	0	,,
36	1p	2p	1p	Mosaic
37	1p	2p*w*, 2p	1g	Leaf-roll
38	(p)	10p	1p, 1g	,,
39	0	1p*w*, 8p	2g	,,
40	(p)	5p	(p)	,,
41	(p)	9p	1g	,,
42	1p*w*, 1p, 1g	2p*w*, 7p	(p)	,,
43	1p	13p	1g	Missing
44	0	1p*w*, 4p, 1g*w*	0	Leaf-roll
45	1p	13p	(p)	,,
46	–	6p	(p)	,,
47	–	(p)	0	Missing
48	1p	5p	1g	Leaf-roll
49	–	1p*w*, 5p	(p)	,,
50	0	2p	(g)	,,

Table I (*cont.*)

Plant	Aphides present on three leaves on 14. vi. 35	28. vi. 35	26. vii. 35	Health of progeny 1936
51	0	13p	(g)	Leaf-roll
52	1p*w*	1p	0	,,
53	(p)	1p	(p)	,,
54	–	1p*w*, 3p	(g)	,,
55	–	1p, 4g	3p	,,
56	1p	1p	3p	,, +interveinal mosaic
57	–	6p	2g	,,
58	–	20p	2p	,,
59	–	5p	(p)	,,
60	0	1p	2p	,,
61	0	15p	(p)	,,
62	0	3p	0	,,
63	1p	16p	6p, 2g	,,
64	1p	24p	(p)	Missing
65	0	7p	(p)	Leaf-roll
66	–	(g)	(p)	,, (one tuber)
67	0	3p	3p	Healthy
68	–	–	1p	Leaf-roll
69	1p	6p, 4g	(p)	,,
70	1p	12p, 6g	1g	,,
71	0	5p, 1g	1p	Missing
72	0	6p, 2g	(g)	Leaf-roll
73	0	4p, 2g	(p)	,,
74	0	1p*w*, 1p	11p	,,
75	1g, (p)	3p	(p)	Healthy
76	0	4p	(p)	Leaf-roll
77	0	2p, 1g*w*, 1g	(g)	,,
78	0	6p	1p	,,
79	0	12p, 3g	1g	,,
80	0	(p)	1p, 2g	,,
81	1p	2p, 5g	0	,,
82	0	3p	0	,,
83	–	(p)	0	,, (one tuber)
84	1p	1p*w*	1g	Healthy
85	–	6p, 3g	(p)	Leaf-roll
86	0	1p, 4g	6p	,,
87	1p*w*	1g, 5p	(p)	,,
88	1p*w*	7p	1g	,,
89	–	3p, 3g	(g)	,,
90	1p	11p	0	,,
91	1p*w*	5p, 3g	3g	,,
92	–	5p	(p)	,,
93	0	5p, 6g	1p	,,
94	1p	15p, 1g	(p)	,,
95	–	13p, 2g	1p	,,
96	1p	1p	(p)	,,
97	1p*w*, 1p	(p)	(p)	,, (one tuber with interveinal mosaic)
98	0	9p, 4g	(p)	,,
99	0	2p	11p	,,
100	–	3p	0	,,

p = *Myzus persicae* (wingless). p*w* = *Myzus persicae* (winged). g = *Macrosiphum gei* (wingless). g*w* = *Macrosiphum gei* (winged). Brackets, e.g. (p) = *M. persicae* present on plant but not on the three leaves taken for count. – = Plant not above ground.

almost every case all four tubers from each unit plant showed symptoms of infection. Eighty-three out of the ninety-three plants tested (seven isolated plants being missing at lifting time) had the progeny infected with leaf-roll, three other plants had leaf-roll and interveinal mosaic, one had leaf-roll together with simple mosaic and there was one case of simple mosaic alone, leaving only five plants with healthy progeny.

This result was surprising in view of the fact that previously (Davies & Whitehead, 1935), when winged aphides had been collected on arrival on healthy stocks at this centre and were transferred to the laboratory, it was found that only a negligible number were infected with virus diseases; this was confirmed in 1935 (cf. p. 138 below). It would appear, therefore, that whereas the initial migrants arriving on potatoes are largely free from virus infection, there is a considerable spread of disease by winged forms at a later date. The uniform infection of all, or nearly all, tubers on each plant also points to such spread occurring fairly early in the growing season, though no doubt it is continued by subsequent winged generations produced on the potato crop. At this centre the nearest source of virus infection was a crop of Arran Comrade potatoes, *ex* Scotland, some 40 yd. distant from the isolated plants and containing 5 % leaf-roll. On a neighbouring farm 3·8–6·4 % of leaf-roll occurred in different varieties.

Centre II (*Ty Mawr, Abergele, Denbighshire*)

This centre, in common with the last one, had been discarded for seed-potato production, under the North Wales Scheme, owing to the rapid spread of virus infection in the stocks. The index figure of the aphis population since 1928 was as follows: 1928 (146, all spp.), 1929 (354, all spp.), 1930 (701, *M. persicae*), 1931 (130, *M. persicae*), 1932 (525, *M. persicae*), 1933 (520, *M. persicae*), 1934 (no record), and 1935 (123, *M. persicae*).

At this centre, also, the general crop of potatoes (about a quarter of an acre) was about 40 yd. away from the nearest isolated plant and in the same field. This main crop contained 4 % leaf-roll and 1 % mosaic. Sixty half-tubers were planted in a mangold crop and at the same intervals as at centre I; the control halves being grown, as before, at the College Farm. Two of the experimental plants were missing at lifting time, and the complete record of the remaining fifty-eight plants is given in Table II.

Counts of the aphis population were taken on only two occasions, since it was found on the second date that practically all plants were

Table II

Aphis population on isolated plants and evidence of virus transmission

Centre II (Ty Mawr, Abergele, Denbighshire). Potatoes isolated in mangold crop, with ¼ acre farm potato crop in same field, distant 40 yd.

Plant	Aphides present on three leaves on 27. vi. 35	Aphides present on three leaves on 25. vii. 35	Health of progeny 1936
1	1p	(p)	Mosaic
2	1p	(p)	,,
3	–	(p)	Leaf-roll + mosaic
4	1p	2p	Mild mosaic
5	1p	(p)	,,
6	1p, 2g	(p)	Leaf-roll
7	–	(p)	Crinkle
8	1g	5p	,, (two tubers)
9	0	(p)	Mild mosaic
10	0	(p)	,,
11	1p*w*	(p)	Crinkle
12	1p*w*	7p	Leaf-roll
13	0	(p)	Healthy
14	4p, 2g	(g)	,,
15	5p, 1g	(p)	,,
16	1p	(p)	,,
17	(p)	(p)	Mild mosaic
18	(g)	1p	,, (one tuber)
19	-	1p	Healthy
20	1p, 1g	(p)	Crinkle
21	–	(g)	Leaf-roll + interveinal mosaic
22	0	1p	Healthy
23	(1p*w*)	28p	,,
24	1p	(p)	,,
25	1p, 1g	1p	Crinkle (one tuber)
26	1p	1p	Healthy
27	3p	(p)	,,
28	2p	1p	,,
29	1p, 1g	2p	,,
30	0	3p	Mild mosaic (one tuber)
31	(p)	3p, 1g	Healthy
32	1p, 1g	(p)	,,
33	1p	2p	,,
34	1p	1p, 3g	,,
35	3p, 1g	1p, 1g	,,
36	3p	(p)	Crinkle (one tuber)
37	5p	1p	,, (one tuber)
38	0	(p)	Mild mosaic (one tuber)
39	(p)	6p	Healthy
40	1p	0	,,
41	1p	1p	,,
42	2p	4p	,,
43	1p	(p)	,,
44	0	(p)	,,
45	1p	2p	,,
46	0	0	,,
47	3p	(p)	,,
48	(p)	1p	,,
49	2p	3p	,,
50	4p, 1g	(p)	Crinkle (two tubers)
51	0	0	Healthy
52	3p	9p	,,
53	0	(p)	,,
54	0	(p)	,,
55	1p*w*	(p)	,,
56	1p*w*	(p)	,,
57	1p, 1g	(p)	,,
58	(p)	(p)	Leaf-roll

Symbols as for Table I.

infested with *M. persicae*. On 27 June, 75·9 % of the plants above ground were infested with *M. persicae*, and at least 22·2 % with *M. gei*. On 25 July, 91·3 % of the isolated plants carried *M. persicae*, so that the ease with which winged aphides detect and colonize such plants was again very apparent.

The progeny of the experimental plants was collected, stored and planted at the College Farm as described for Centre I, the condition of health being recorded in the last column of Table II. It will be observed that out of fifty-eight isolated plants the progeny of twenty-three (or 39·6 %) proved to be infected with one or more virus diseases. Two of these showed symptoms of leaf-roll alone, two showed leaf-roll and mosaic, eight were infected with a crinkle complex, two with mosaic alone, and eight with a mottling probably representing a very mild infection with a form of mosaic. Here also, as at centre I, there was evidence of considerable spread of virus diseases (in this case mostly of the mosaic type) when only a small crop of potatoes occupied the same field.

Centre III (*Hawarden, Flintshire*)

At this centre the half-tubers were planted in a mangold crop as at the two previous centres, with the general farm crop of potatoes growing in the same field but distant at least 100 yd. from the nearest experimental plants. No detailed information of the aphis population over a number of years was available, though aphides are common in the surrounding district and the index figure was frequently over 500 of all species per 100 leaves. Nor was there any exact knowledge of the rate of spread of virus diseases though it was believed to be fairly rapid and it is the general custom to make frequent changes in the seed potato stocks grown. A count of the aphis population on the experimental plants was not made, but when the progeny of the forty-eight isolated plants was grown at the College Farm in 1936, eight of them (16·7 %) were shown to have contracted leaf-roll at Hawarden in 1935.

Centre IV (*Dyserth, Flintshire*)

As at the last centre there was no previous knowledge of the aphis population or of the rate of spread of viruses when this centre was selected. It is, however, only a few miles distant from centre I and with very similar topographical conditions. The aphis population in general is high, and it is a common practice for growers to change their seed potatoes frequently. One important point of difference, however, from

the first three centres described, was the degree of isolation obtained for the experimental plants, these being planted in a crop of sugar beet and at a distance of at least a quarter of a mile from the nearest farm crop of potatoes. This latter crop contained some virus-infected plants, but the percentage was unfortunately not recorded.

When an aphis count was taken on 27 June, ninety-five out of the 100 isolated plants were found to be already infested with *Myzus persicae*. Unfortunately some of the plants were inadvertently removed before lifting time at this centre but the records of the sixty-seven remaining plants, given in Table III, suffice to indicate the nature of the aphis population. Sixty-three of these plants were infested with

Table III

Aphis population on isolated plants and evidence of virus transmission

Centre IV (Dyserth, Flintshire). Isolated tubers grown in sugar-beet crop; no potatoes within a quarter of a mile

Plant	Aphides on 27. vi. 35	Health of progeny 1936	Plant	Aphides on 27. vi. 35	Health of progeny 1936
1	1p	Healthy	35	1p*w*	Healthy
2	3g (p)	,,	36	2p, 1g	,,
3	(p)	,,	37	3p	,,
4	(p)	,,	38	(1p*w*)	,,
5	2p*w*, 8p	,,	39	(p)	,,
6	1p	,,	40	7p	,,
7	1p*w*, 3p	,,	41	1g	,,
8	1p	,,	42	1p*w*, 10p	,,
9	(p)	,,	43	3g, (p)	,,
10	1p	Leaf-roll	44	3p*w*, 18p, 2g	,,
11	1p*w*, 1g	Healthy	45	2p, 1g	,,
12	1p	Leaf-roll	46	(g)	,,
13	(p)	Healthy	47	4p	,,
14	0	,,	48	3p	,,
15	2p*w*, 1p	,,	49	10p	,,
16	1p	,,	50	3p, 6g	,,
17	4p, 1g	,,	51	8p, 7g	,,
18	(p)	,,	52	8p, 4g	,,
19	1p	,,	53	4p	,,
20	(p, g)	,,	54	15p, 1g	,,
21	(p)	,,	55	(p)	,,
22	2g (p)	,,	56	3p	Leaf-roll
23	1g	,,	57	4p	Healthy
24	1p	Leaf-roll	58	2p, 2g	,,
25	1p	Healthy	59	5p	,,
26	1p, 1g	,,	60	4p, 2g	,,
27	1g (p)	,,	61	1p*w*, 8p	,,
28	(1p*w*)	,,	62	3p	,,
29	5p, 2g	,,	63	4p, 1g	,,
30	6p, 1g	Leaf-roll	64	2p, 1g	,,
31	1p*w*, 7p, 1g	Healthy	65	1p	,,
32	4p, 4g	,,	66	(p)	,,
33	3p, 2g	,,	67	1p*w*, 2p	Mosaic
34	2p, 2g	,,			

Symbols as for Table I.

M. persicae on 27 June and twelve *winged* forms were seen although only three leaves per plant were examined; twenty-seven plants at least were infested with *Macrosiphum gei*. These figures present ample evidence to show that when potatoes are planted singly, in isolation in a root crop, and with no other potatoes within a distance of a quarter of a mile, winged aphides have no difficulty in detecting and colonizing the single unit plants.

When the health of the progeny is examined in the record shown in Table III, it is clear that the spread of viruses was much less than at the first three centres. All four centres were located in districts definitely regarded as unsuitable for seed-potato production by reason of the rapid spread of viruses, and the data now presented give some indication of a correlation between virus spread in such localities and the distance between potato crops. It is evident also that in such districts it will be difficult to secure a degree of isolation sufficient to ensure the maintenance of health in seed stocks. At centre IV, although the isolation was better than could ordinarily be obtained, and notwithstanding that aphis infestation was low during 1935 in this district, six out of the sixty-seven isolated plants (8·9 %) had contracted virus diseases (five having leaf-roll, and one showing mosaic) in that year.

Centre V (College Farm, near Bangor, Caernarvonshire)

This centre is in an entirely different district from those described above. It is more humid and low-lying; being exposed to the open sea one mile to the north and backed by mountains within a mile to the south. The aphis population at this centre is always much lower than in the eastern districts where the first four centres are located, but in certain seasons the population is fairly high with an index figure of about 300 aphides per 100 leaves examined. A museum of virus infected potatoes is maintained each year in the potato field and the rate of spread of viruses is appreciable, though not so rapid as in the eastern districts to which reference has been made. The district can be regarded as intermediate between east and west both as regards aphis population and rate of virus spread.

The experimental half-tubers were planted in isolation in a crop of mangolds, about 40 yd. to the east of the general farm crop of potatoes and the virus museum; all being in the same field. The mangold crop with its isolated potato plants was on a steep slope with an easterly, exposed aspect. The records of aphis infestation and the spread of disease as shown in the progeny are given in Table IV. In addition,

since there was a heavy attack of the capsid *Calocoris norwegicus* on the first row (plants 1–33), an asterisk indicates severe damage to the plant.

Table IV

Aphis population on isolated plants and evidence of virus transmission

Centre V (College Farm near Bangor, Caernarvonshire). Isolated plants in mangold crop and in same field as heavily infected potatoes

Plant	Aphides present on three leaves on			Health of progeny 1936
	21. vi. 35	2. vii. 35	25. vii. 35	
1	0	0	1p (par)	Healthy
2	0	3p	1p	,,
3	0	1p*	0	,,
4	0	2p*	0	,,
5	0	(p)*	0	,,
6	0	0*	0	,,
7	0	0	0	,,
8	0	1p*	1p (par)	,,
9	1p	0*	0	,,
10	(p)	(p)*	1p	,,
11	(p)	0*	0	,,
12	0	1p*w*	0	,,
13	0	2p	0	,,
14	1p	1p*	0	,,
15	(p)	0*	0	,,
16	0	(p)	0	,,
17	0	0*	0	,,
18	0	0*	0	,,
19	(p)	1p*	0	,,
20	0	0*	0	,,
21	0	1p*w**	0	,,
22	(p)	0*	1p (par)	,,
23	0	0*	0	,,
24	0	1g*	0	,,
25	0	0*	1p	,,
26	0	1g*	0	,,
27	0	0*	0	,,
28	0	0*	0	,,
29	0	0*	0	,,
30	0	0*	0	,,
31	1p	0*	0	,,
32	0	0*	0	,,
33	0	0*	0	,,
34	(p)	(p)	–	,,
35	0	0	–	,,
36	0	1p	–	,,
37	(p)	(p)	–	,,
38	0	0	–	,,
39	0	0	–	,,
40	0	1p (par)	–	,,
42	0	0	–	,,
43	0	0	–	,,
44	0	0	–	,,
45	0	1p	–	,,
46	1p*w*	18p	–	,,
47	0	1p	–	,,

* = plant severely damaged by *Calocoris norwegicus*. (par) = parasitized. Other symbols as in Table I.

Table IV (*cont.*)

Plant	Aphides present on three leaves on 21. vi. 35	2. vii. 35	25. vii. 35	Health of progeny 1936
48	0	0	–	Healthy
49	1p	3p	–	,,
50	1p	1p	–	,,
51	0	0	–	,,
52	1g*w*	1g (par)	–	,,
53	1p	2p	–	,,
54	(p)	4p	–	,,
55	0	0	–	,,
56	0	1p	–	,,
57	0	0	–	,,
58	0	0	–	,,
59	0	2p	–	,,
60	1p	0	–	,,
61	0	1p	–	Leaf-roll
62	0	0	–	Healthy
63	0	0	–	,,

The aphis infestation of the isolated plants was markedly lower than at the centres previously described although thirty-four out of the sixty-three plants had *Myzus persicae* present at one or other date of examination. This lower infestation was due to the fact that there was only a negligible migration of winged aphides, which was confirmed by the data obtained in collecting winged aphides daily in the nearby mechanical trap (Davies, 1935). The spread of virus diseases to these isolated plants was correspondingly slight, for only one plant yielded infected progeny; a result which was a little surprising in view of the large number of diseased plants growing in the same field. Other experiments in the same field confirmed the view that the lack of virus transmission was due to scarcity of winged migrants. A small plot of 100 healthy potatoes was grown in isolation within the mangold crop, primarily for the purpose of studying the aphis population and as a control for spraying trials. The aphis population on this unsprayed, control plot proved to be very slight, not exceeding ten per 100 leaves, but it was decided to take a sample of the tubers for planting in 1936 in order to discover the amount of virus transmission occurring under these conditions. This was compared with a plot of similar size growing in the general crop of potatoes alongside the virus museum. The progeny of the isolated plot in the mangold crop showed 1·1 % infection only; indicating a slight migration of winged forms to the crop, for only such forms had access to the plants. On the other hand, 10·74 % of the plants adjacent to the virus-infected material became infected, this representing disease transmission to be ascribed both to Alatae and Apterae. There

was no evidence that *Calocoris norwegicus* was a vector of virus diseases under field conditions.

Centre VI (Madryn, Bodfean, Caernarvonshire)

This centre, and the following one, were selected in the districts where there had been no increase in virus infection among potato stocks since they were introduced in 1928. At each centre fifty half-tubers were planted in isolation in mangold crops with the general farm crop about 40 yd. distant in the same field. In view of the fact that the progeny of the general crops had showed no increase in virus infection during 8 years, it was only necessary to seek further confirmation of a direct correlation between this lack of transmission and the number of winged aphides present. It was only possible to make a single aphis count, but this sufficiently indicates the slight nature of the infestation, although the sheltered position of this particular field would permit greater migration than is general in this district. The progeny from half of the isolated plants only was removed to the College Farm for subsequent planting, and it will be seen from Table V that no disease occurred in this progeny in 1936. This failure to transmit was not due to an entire absence of infected plants in the main crop, for, from some unexplained cause, the infection in this crop increased from the normal 0·5 to 1·15 % in 1935.

Table V

Aphis population on isolated plants and evidence of virus transmission

Centre VI (Madryn, Bodfean, Caernarvonshire). Isolated plants in mangold crop. Two acres of farm crop of potatoes in same field. Progeny taken from only twenty-five plants

Plant	Aphides on 10. vii. 35	Health of progeny 1936	Plant	Aphides on 10. vii. 35	Health of progeny 1936
1	0	Healthy	14	0	Healthy
2	1p*w*	,,	15	0	,,
3	0	,,	16	0	,,
4	0	,,	17	0	,,
5	2p	,,	18	1g	,,
6	0	,,	19	1p	,,
7	(1p*w*)	,,	20	0	,,
8	0	,,	21	0	,,
9	0	,,	22	1p	,,
10	2p	,,	23	0	,,
11	0	,,	24	(p)	,,
12	2p	,,	25	0	,,
13	1p*w*	,,			

Symbols as for Table I.

Centre VII (*Hendre, Aberdaron, Caernarvonshire*)

This centre is one of the best seed-producing farms; it is low-lying, exposed, and stocks have been grown without increase of virus infection since 1928, the figure being 0·52 % in 1935. The aphis population has always been very low and the index figure of *M. persicae* in mid-July has not, normally, exceeded twenty per 100 leaves. This index figure since 1928 has been as follows: 1928 (negligible, all spp.), 1929 (24, all spp.), 1930 (5, *M. persicae*), 1931 (58, *M. persicae*), 1932 (44, *M. persicae*), 1933 (82, *M. persicae*), 1934 (26, *M. persicae*), 1935 (12, *M. persicae*).

It will be seen from Table VI that the aphis infestation of the isolated plants at this centre was very slight and that there was no evidence of transmission of any virus having occurred in 1935, as judged by the state of health of the plants grown from four tubers taken from each of the plants in that year.

Table VI

Aphis population on isolated plants and evidence of virus transmission

Centre VII (Hendre, Aberdaron, Caernarvonshire). Isolated plants in mangold crop. Farm crop of potatoes in same field of two acres. Progeny taken from only twenty-five plants

Plant	Aphides on 10. vii. 35	Health of progeny 1936	Plant	Aphides on 10. vii. 35	Health of progeny 1936
1	0	Healthy	14	0	Healthy
2	2p	,,	15	0	,,
3	0	,,	16	0	,,
4	0	,,	17	1p, 1g	,,
5	0	,,	18	1p	,,
6	0	,,	19	0	,,
7	1p	,,	20	0	,,
8	0	,,	21	0	,,
9	0	,,	22	0	,,
10	0	,,	23	1p*w*	,,
11	0	,,	24	0	,,
12	1g	,,	25	0	,,
13	0	,,			

Symbols as for Table I.

Development of the aphis population on isolated plants compared with that on the nearest potato crop

The development of the aphis population on isolated plants and on a general crop of potatoes can best be compared by studying the data at centre I (Berthymaen), presented in Table VII.

All the isolated plants infested with *M. persicae* on 14 June were still infested with this species on 28 June (cf. Table I), and even on 26 July 69·5 % of those initially infested still had this species present.

This indicates that colonization was successful following the initial migration of the winged migrants.

Table VII

Infestation of aphides on isolated plants and (in brackets) on the general farm crop of potatoes at Berthymaen, 1935

Date	14 June	28 June	26 July	25 August
No. of plants	75*	98 (50)	98 (50)	99 (50)
Percentage infested with *M. persicae*	62*	98·9 (98)	63·6 (90)	25 (–)*
No. of *M. persicae* per 100 leaves	20·8*	164 (294)	30·3 (210)	5·9 (79·2)
No. of winged *M. persicae* per 100 leaves	8·4*	7·8 (4·6)	0·0 (0·0)	0·0 (0·0)
No. of *M. gei* per 100 leaves	1·3*	22·3 (122)	13·4 (232)	3·8 (89·6)
No. of winged *M. gei* per 100 leaves	0·0*	1·02 (1·3)	0·0 (0·0)	0·0 (0·0)

* No aphis count made on general farm crop.

The increase in the population of *M. persicae* was quite rapid since within a fortnight the index figure had increased from 20·8 to 164 per 100 leaves. But there was also a rapid decline (cf. Table VII), since by 26 July the index figure had fallen to 30·3, and on 25 August it was only 5·9 for *M. persicae* per 100 leaves. The continuous observation maintained on these plants at Berthymaen clearly showed that this reduction was due to the activities of predators and parasites which, with the limited environment of the isolated plant, took a heavy toll of the aphides. There was evidence also that thunder-rain reduced the population on the exposed isolated plants. The rapid increase in the total population of *M. persicae* and its subsequent decline was also evident at the other centres.

A comparison with the aphis population on the general potato crop in the same field shows the latter to be higher, the index figure on 28 June being 294 *M. persicae* compared with the 164 of the same species on the isolated plants. Further, the infestation on the general crop was better maintained, for on 26 July the index figure in the crop was still 210 compared with the 30·3 *M. persicae* per 100 leaves of the isolated plants. From this date, however, the population declined rapidly until only 79·2 *M. persicae* could be counted per 100 leaves on 25 August.

The period during which winged aphides were collected on the isolated plants is of some interest, since it is believed to indicate the time during which the initial migrants were arriving and actively moving from plant to plant. At three centres a sequence of records was obtained of the winged forms taken on the isolated plants. The average number per plant was as follows: Centre I: 14 June, 8·4; 28 June, 7·8; 26 July, nil; 25 August, nil. Centre II: 27 June, 2·5; 25 July, nil. Centre V:

21 June, 0·5; 2 July, 1·0; 25 July, nil. This arrival of winged migrants on potatoes during June and the decline in numbers at the end of July has been noted for several seasons. It is not until after this period that winged forms, which have been produced on the potato crops, begin to appear among the aphis colonies.

The infestation of *Macrosiphum gei*, although slight, presented points of some interest. It was never high on the isolated plants at any centre, and, even at centre I, was only 22·3 per 100 leaves on 26 July, declining to 3·8 by 25 August. In the general farm crop of potatoes in the same field, however, the corresponding index figures of population were: 122 on 28 June, 232 on 26 July, and 89·6 on 25 August. This lower infestation of *M. gei* on the isolated plants as compared with that of the general farm crop was also apparent at centres II and IV; the index figures on the isolated plants at these centres being 2·8 and 25·3 per 100 leaves respectively, whereas in the general crop the figures on the same day were 120 and 70 per 100 leaves respectively.

Discussion

(*a*) *Colonization*

The remarkable ease with which winged *Myzus persicae* detect and successfully colonize isolated potato plants has been clearly demonstrated; for instance, at centres I, II, and IV, 98·9, 91·3, and 94·0%, respectively, of these plants were visited by winged migrants of this species before the plants were 6 in. high. Since this infestation took place in a season when the aphis population in these districts was unusually low, it can be inferred that *M. persicae* will migrate to and populate any potato crops growing even in relative isolation in these districts.

The distance over which winged aphides will migrate is difficult to discover because there is no easy way by which marked winged aphides can be attracted after release. There is some evidence (cf. Davies, 1936) that winged aphides can be carried involuntarily over considerable distances, but this enforced *transportation* should not be confused with *voluntary migration*, which is the type of migration associated with the normal colonization of host plants. The records of infestation by winged aphides of the exceptionally isolated plants at centre IV are, therefore, particularly interesting in this connexion. At this centre the isolated plants, growing in a sugar-beet crop, were distant at least a quarter of a mile from the nearest potatoes; thus fixing this distance as the minimum flight of the virulent migrants. The root field, moreover, was on a plateau

on the reverse slope from the nearest potato crop and facing the direction from which the light, dry breezes usually waft the migrating aphides. It is probable, therefore, that the migrants had covered a distance much greater than the quarter of a mile separating the isolated plants from the nearest potato crop.

(*b*) *Spread of virus infection*

The contrast in the aphis population of potato crops in the eastern districts where spread of viruses is usually rapid, with that of western districts where little if any spread occurs, has been fully established (Whitehead *et al.* 1932; Davies, 1934). It was, however, uncertain whether this was due to a difference in numbers of winged migrants or to a difference in the rate of reproduction of aphides within the crop. The present work shows that there was a very much smaller number of winged migrants at the western centres than at those in the east, and that the possibility of virus transmission must be correspondingly reduced in the west. Investigation carried out by the writers in 1934 (Davies & Whitehead, 1935) had shown that only a minute percentage of winged aphides (i.e. 0·34 %) were already infected with viruses when they arrived on the experimental potato plot which, in point of fact, was located at centre I. This was confirmed in 1935, although only 200 winged *M. persicae* could be collected during a daily search from 15 June to 23 July at this centre, in what proved to be an exceptionally low year of infestation. The winged migrants so collected were despatched to Bangor and isolated in batches of five each on healthy half-tubers. Although the numbers were small the results were in keeping with those of 1934 for none of the aphides proved to be infected with a virus. It is interesting, however, to note that of twenty-nine winged *M. persicae* taken from "ground keepers" on 14 June at least two were infected with leaf-roll. It is important to remember that this virtual freedom from virus infection of the initial migrants to potatoes was demonstrated at a centre where virus infection in a crop had increased in *two years* from 0·23 to 33·0 % (Whitehead *et al.* 1932). It would seem, therefore, that this rapid spread of viruses must have been due to aphis movements within the crop itself, but whether by winged forms or Apterae was not determined. The present results, by giving the amount of transmission to isolated plants, implicate winged forms only, and the high percentage of plants infected suggests that transmission was due to short, frequent movements of winged aphides within the crop. At centre I, 94·6 % of the plants became infected, and even at centre II, with only a small plot

of potatoes in the field, infection was carried to 39·7 % of the isolated plants. At centre III this percentage fell to 16·7, due, it is presumed, to the greater distance (100 yd.) from which infection had to be carried. These facts confirm the opinion of the writers that the spread of virus diseases from crop to crop is due mainly to the relatively small numbers of winged forms still migrating after feeding on many potatoes, or to winged aphides, again usually fairly small in numbers, produced later in the season on potato crops.

(*c*) *Protection from virus infection*

The seven centres at which the present work was carried out fall into three reasonably distinct groups. The first group consists of centres VI and VII where both winged aphides and sources of virus infection were exceptionally small in number. Under such conditions the problem of protection is simple and need only be such that Apterae are prevented from crawling from plant to plant. The second group is represented only by centre V, where sources of infection are extremely numerous and the possibility of adequately protecting a crop from infection will be determined by seasonal factors which regulate the alate aphis population. In the year under consideration (1935) very low counts of Alatae were made throughout North Wales and, at centre V, the number had steadily declined from a "peak" infestation in 1933. This low alate population was reflected in the small amount of virus transmission (i.e. 1·6 %) which occurred at centre V, notwithstanding the numerous sources of infection within 40 yd. of the isolated plants. Even a small, compact plot of potatoes at a similar distance from infectors was only infected to the extent of 1·1 %, whereas a plot adjacent to infectors, and so accessible to crawling Apterae, had disease transmitted to nearly 11 % of the plants. In a year unfavourable for the development of aphides, therefore, the proximity of heavily infected potato stocks had little effect upon the spread of virus diseases, and a fairly high degree of protection was afforded by a distance of 40 yd. from infectors. On the other hand, in seasons favourable for aphis development (as in 1933) the population at centre V approximates to that found in 1935 at centres I–IV, and would be included, with them, in the third group, in which Alatae are numerous and sources of infection are not negligible. Under such conditions a distance of 40 yd. from infected plants gives little or no protection to the isolated potatoes. It is at centre IV, however, that the difficulty of adequately protecting a crop becomes most apparent. Here, relatively large numbers of winged aphides were in movement and disease was

transmitted to practically 9% of the isolated plants. The latter were at a distance of a quarter of a mile from the nearest potato crop, and both wind direction and topographical features, as well as the unusually low aphis population (for that centre) operated against any great amount of transmission of disease. Moreover, it will be realized that the likelihood of infected winged aphides passing from plant to plant was probably less than would have been the case with adjacent plants of a general crop, whilst wingless forms, produced on an infected isolated plant would have little chance of transferring the infection to other plants in isolation.

It can safely be said that any practicable means of protection of a healthy crop of potatoes, based on distance from a partially infected stock, would fail under conditions approximating to those found at centres I–IV, and that effective protection would be still more difficult in arable areas where potatoes are an important crop in the rotation. These facts are of particular importance in framing regulations for the improvement of the health of seed potato stocks. The present work shows that it is not enough merely to require minimum distances of isolation between stocks, without taking cognisance of aphis population or of the state of health of neighbouring crops. The criterion should obviously be the risk of infection of the stock seeking certification, and this is not solely, or even mainly, a question of distance. What, in the opinion of the writers, should be aimed at is the segregation of districts (or farms) into classes based on a survey of all these factors, with appropriate conditions for certification of seed stocks in each case; some such scheme as this was suggested by the writers and Mr J. F. Currie in 1932 (Whitehead *et al.* 1932). Uniformity of regulations over large areas of country is especially to be deprecated, for the distance of isolation required will be unnecessarily great in really good seed producing districts and entirely ineffective elsewhere.

Summary

1. The investigations were planned to ascertain the extent of the migrations of winged aphides, their ability to detect individual plants, the development of aphis population following colonization, and the extent to which virus diseases may be transmitted to isolated plants by winged aphides.

2. Plants were isolated in root crops on seven farms and at varying distances from probable sources of virus infection. Four farms were located in districts where aphis population is usually high and viruses spread very rapidly; two in districts where both aphides and spread of

viruses are minimal, whilst one farm could be regarded as intermediate in both respects.

3. Evidence is submitted to show that winged aphides have no difficulty in detecting and colonizing isolated plants. Their numbers were large at the four eastern centres and very small at the three western centres. In all cases the aphis population on isolated plants was less than that of the nearest general crop and suffered more from predators and parasites.

4. It is shown that isolated plants can be reached by migrants from a distance of at least a quarter of a mile, and probably much further. Additional evidence is given that these initial migrants introduce little virus infection to potato crops from extraneous sources. The importance, however, of the later movements of winged aphides, whether initial migrants or those subsequently produced on potatoes, in spreading viruses from crop to crop, is emphasized.

5. The practical aspects of protecting potato stocks is discussed under conditions of (*a*) heavy infestation by winged aphides and proximity of partially diseased crops; (*b*) minimal winged aphis infestation where numerous sources of virus infection occur in neighbouring crops; and (*c*) where both aphis infestation and sources of infection are minimal. It is considered that regulations for the improvement of health in seed-potato stocks should take cognisance of these various possibilities.

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FACTORS AFFECTING THE FLUCTUATIONS IN THE POPULATION OF *TOXOPTERA AURANTII* BOY. IN PALESTINE

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(With 4 Text-figures)

Introduction

Aphids become very troublesome in Palestine only on occasions when weather conditions have been exceptionally favourable for their development and reproduction. Otherwise they are of little importance to the farmers. Thus *Toxoptera aurantii* Boy. is seldom found in the *Citrus* grove throughout the summer and is scarce in the fall and winter. It becomes numerous only in the spring for a short time, and, as a rule, the damage caused is of little importance. Occasionally, however, without warning, an unexpectedly serious infestation takes place, when the farmers become alarmed and seek to employ methods of control. But the control measures are thought of when it is too late—after the damage has been done, when spraying brings no benefit and, on the contrary, may harm the trees. For these reasons it was found to be of interest to study the factors underlying such sporadic outbreaks and to analyse them with a view to forecasting possible infestations. The following is an attempt to analyse some of the factors controlling the increase and decrease in population of the *Citrus* aphis.

Forms of the aphis

Like many other aphids in Palestine, *Toxoptera aurantii* Boy. can reproduce continuously throughout the year by viviparous parthenogenesis, no sexual reproduction taking place. Although the insect was bred for over 3 years no sexual, egg-laying females were obtained in the broods nor were any discovered in the grove. The forms most prevalent are the agamic apterous and agamic alate females. The ratio between the numbers of the two forms depends upon external factors as well as food conditions, and a more extended discussion of this subject is given

elsewhere (Rivnay, 1937). An additional alate form was discovered in the course of breeding. This is the male of the species, perhaps a vestigial member of the sexual forms.

Rate of development

The data presented below were obtained by breeding the species in the laboratory.

The individual was bred on a soft twig, one end of which was placed in water and the other end in a short test-tube. The development of the

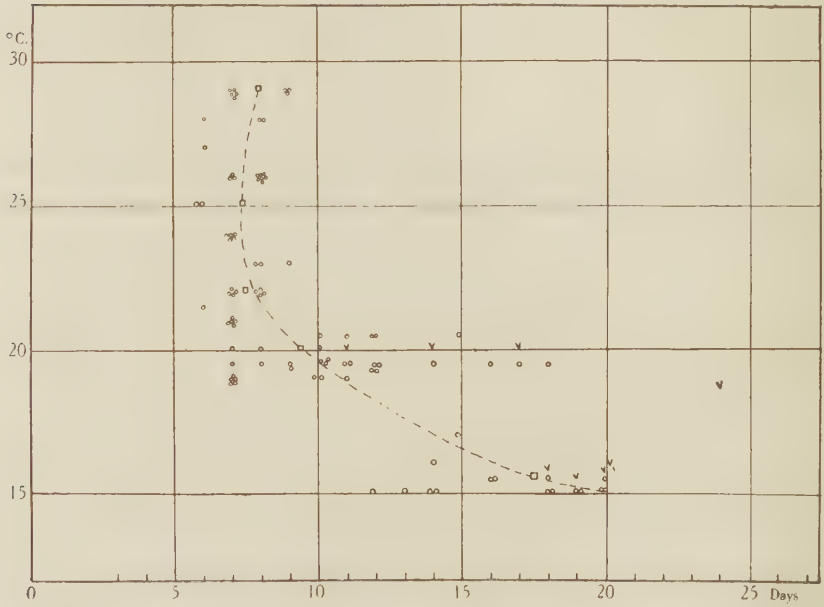

Fig. 1. Curve presenting relationship between temperature and rate of development of the aphis *Toxoptera aurantii*. Each dot represents average period of development of a few apterous females; the wedge represents that of a few males.

apterous agamic female from its birth until it gives birth lasts, under favourable conditions of temperature, about 6–7 days. Fig. 1 presents the rate of development of the apterous agamic female at various degrees of temperature, each dot representing the average record of one breeding, which consisted of three or five females. It is noticeable that the temperature at which development is shortest is between 22 and 25° C. This temperature proved to be the most favourable also for reproduction. At 18–22° C. development is not much slower than at the optimum

temperature. The development may then be from 7 to 12 days, the average being a period of 9½ days. This has a great bearing as a factor in the problem of infestation. At the temperature of 15° C. the development of the aphid takes about 2 3 weeks, and below this temperature the development is so slow that it is of little importance as a factor of infestation. If the temperature rises above the optimum of 22–25° C., development is slightly retarded (see Fig. 1).

Reproduction

The female aphis begins to reproduce almost immediately after she has reached the adult stage and continues to bear young until 2 or 3 days before she dies. During the course of this time a peak in the curve

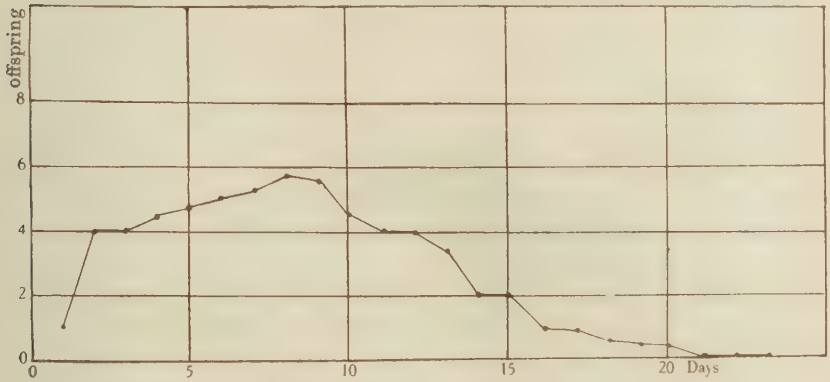

Fig. 2. Curve representing the rate of reproduction of the *Citrus* aphis *Toxoptera aurantii* at a temperature of 22° C.

(Fig. 2) which presents the rate of reproduction may be distinguished, covering a short period when the rate of reproduction of this particular female is at its maximum. After this the rate of reproduction decreases gradually until only one nymph is born in approximately every 2 days. The curve in Fig. 2 represents the rate of reproduction based on the average taken from ten individuals at a temperature of 22° C. It is noticeable that at this temperature the maximum number of young that one female bore in 1 day was approximately five to six. However, in the course of the several breedings some individuals bore even seven to eight young in 1 day. The rate of reproduction decreases as the temperature falls. Thus, at a temperature of from 10 to 13° C., one nymph was borne by one female each day. No reproduction took place at 7° C. At

a temperature above the optimum, reproduction diminishes, so that at a temperature of 33° C. individuals do not reproduce or else they bear only very few young, approximately one nymph a day during their short life. At a temperature of 34–35° C. no young were born at all.

When the temperature and humidity are favourable one female may bear as many as eighty offspring during her lifetime. On the average the maximum production of one female is about seventy young, which takes place at the optimum temperature of 22–25° C. With decrease or increase of the temperature the reproductive power of the female also decreases (see Table I).

Table I

Temp. in ° C. ...	12·5–14·5	15–18	22	25	28–29	32–33	34–35
No. of females	6	11	11	5	11	4	50
Total no. of their offspring	66	302	656	334	274	29	0
No. of offspring per female	11	27	60	67	25	7	0

Effects of temperature upon mortality

Under favourable conditions and optimum temperature, the apterous female of the *Citrus* aphis may live from 20 to 30 days. In the winter, however, at an average temperature of about 15° C., her life may be prolonged to about 60 days. Temperatures above 25° C. shorten her life to a great extent. Thus, at 28–29° C., the average life of ten individuals was about 10·3, while at 32–35° C. the average length of life was about 6 days; of fifty females, twenty-one died within 3 days, twenty-four within 4–9 days, and only six survived from 10 to 11 days. At 36° C. most of fifty insects died within a day or a fraction of a day, few surviving to the next day and then dying.

Young nymphs of the first and second instars can hardly survive above 30° C. For instance, out of 121 young reared in four breedings at a temperature of 30–32° C., only four individuals survived to reach the adult stage. These were much smaller than the normal size of the species and died within 2 or 3 days, leaving no offspring.

Effects of humidity

Generally the aphids were bred under conditions of high relative humidity. Some breedings, however, were carried out in very dry environments, and it was found that this insect is quite tolerant to external changes of relative humidity as long as it is kept within optimum ranges of temperature and a good supply of food is available. A very low relative humidity may stimulate the development of wings, but

otherwise only slight effects were noticed on the rate of development and reproduction. However, at higher temperatures it was found that a low relative humidity greatly affects the insect and lowers the thermal death-point.

Annual fluctuations in the population of the aphis

The annual fluctuations of the population of the black *Citrus* aphis throughout the year are characterized by a distinct increase towards the end of February and March followed by a sudden decrease in April. This decrease may be so abrupt that not a single living aphid can be found on the trees 10 days after the climax had taken place. Slight reinfestations may appear during the summer months if the weather is exceptionally mild; otherwise, no aphids are to be found until the following fall.

The infestation of the spring 1936, which was quite troublesome, was carefully studied in the groves of the Experiment Station at Rehovoth and is described below. From several reports it is evident that the situation throughout the country was similar to this, and it may be considered as typical of the general situation along the coast of Palestine.

Single colonies of aphids were observed throughout November, December and early January. Towards the end of January and early February they began to be more common, and towards the end of February the colonies increased to such a degree that the insect became a nuisance. Towards the middle of March the pest presented a problem, and, unlike preceding years, *Citrus* growers were making preparations to employ measures of control. However, towards the middle of April the insect diminished very considerably, and about 25 April not a single living aphid was to be found on the trees.

Counts, whereby the annual fluctuations could be presented, were not made for the following reasons: The number of aphids per tree could not give a true picture of the infestation because one single twig on a tree may harbour more insects than another tree where the aphids are distributed on every new shoot. Yet the latter presents a more serious infestation. Again, the counting of infested trees would not give a true picture of the situation because a single small colony on a tree would classify the tree as infested and it would not distinguish it from a tree which was really heavily infested with the pest. It was found more advisable, therefore, to present the curve of fluctuation in terms of four degrees of infestation, namely, (1) no infestation, (2) slight infestation, where single colonies were present here and there, (3) moderate infestation,

where the pest became more conspicuous on a large percentage of trees, and (4) heavy infestation, when every tree had every new shoot infested. In these terms the fluctuation of the aphid population in the spring of 1936 is shown in Fig. 3.

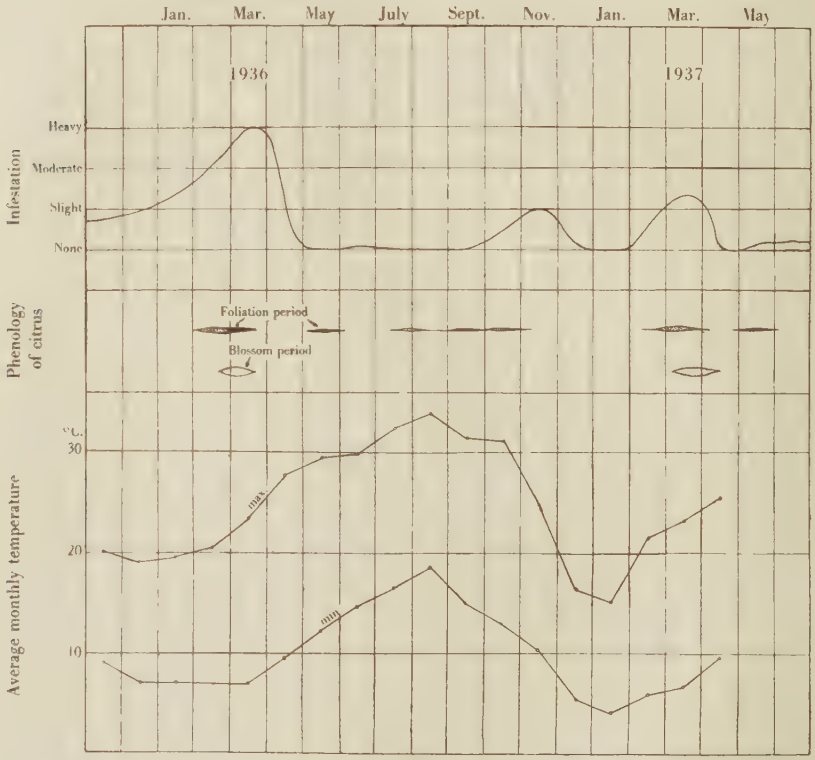

Fig. 3. Graph showing the fluctuation in the population of *Toxoptera aurantii* during the years 1936 and 1937, together with the phenology of *Citrus* trees and the fluctuations in the temperature of the same period in Palestine.

Food as a factor in the increase of population of the aphid

One of the factors responsible for the curves in Fig. 3 is the availability of the food. *Toxoptera aurantii* feeds on very soft, young leaves or on the tips of newly developed shoots. As soon as the leaves become mature and hardened, the aphids leave them. In laboratory experiments, several young nymphs were placed to feed upon mature leaves, but the young crawled away from these leaves in search of better food. When they were

confined to such food they starved within a day or two. On the other hand, when placed upon a soft twig, they always remained and developed thereon. In view of this fact it is quite obvious that the abundance of the aphids depends to a great extent upon the abundance of suitable food, that is, new growth. For this reason the peak of the aphis infestation takes place simultaneously with the new growth of the spring, as may be observed in Fig. 3, i.e. during the month of March. Infestations at other periods of new growth, during the summer, are hindered by unfavourable conditions of temperature as will be discussed below, while the new growth during the months of September and October stimulates and encourages the reinfestation of the grove after the summer.

Temperature as a factor influencing annual fluctuations of the aphis

The temperature during the months of December and January is quite low and insufficient to make possible a great multiplication of the aphis. On the one hand, the food supply during these months is quite scanty and discourages the mass reproduction of the aphis and on the other hand, the temperature itself is unfavourable for its development. The temperature during the nights is far below that at which development and reproduction can take place (below 8–10° C.) and the day temperature is on the average from 12–15° C. At such a temperature the development of the aphis is about three weeks and consequently reproduction is greatly retarded. Thus, a noticeable increase of this insect cannot take place during December, January and early February.

As mentioned above, the optimum temperature for development and reproduction of the aphis is between 22–25° C. As a rule the average day temperature during the month of March in Palestine is within these temperature limits. It is true the nights are still cool, but the temperature during the day is sufficiently high to enable the aphis to produce at least three generations during the month, so that one single individual is capable of giving rise to over 100,000 offspring within the month of March. Hence the sudden increase of the insect during that month. However, this progress is checked during April, the temperature of which is catastrophic for the insect. It has already been stated that at 32° C. the insect is no longer capable of development; at 33–34° C. it no longer reproduces; and at 36° C. it dies within a short period. If this high temperature is coupled with very low atmospheric humidity, death takes place even faster or instantaneously. During April it happens, quite often, that the temperature rises even above this limit. Thus, during

April 1936, on the 12th and 18th days of the month, the maximum temperature in Rehovoth reached 41° C. and the relative humidity fell to 10%; during the 21st to 24th of the same month the temperature was 41–42° C. Inspection in the grove showed that a great percentage of aphids died after the 18th yet a few were still alive, the high temperature apparently not lasting long enough to kill all. However, not a single living aphis was found on the trees after the 25th. No doubt, as a result of the dryness of the atmosphere, many of the young aphids developed into alatae, and when the detrimental temperature set in they were capable of flying off the trees in search of cooler places in the ground. All the apterous forms were no doubt killed, thus leaving no individuals on the trees. The general maximum temperature of that summer in Palestine was too high (33° C.) to allow a re-establishment of the aphis. In fact, throughout the summer months the *Citrus* grove adjacent to the Experiment Station was free from infestation. The first colony of reinfestation was found on 7 October 1936, and consisted of about six mature apterous females and their offspring, the oldest of which were about 4–5 days old. The mature aphids were no doubt borne by an alate individual which emerged from the ground after the weather had cooled somewhat, probably towards the end of September.

Limiting factors

Since it has been pointed out how the annual fluctuations of the temperature bring about the annual fluctuations of the aphis population, it is of interest to know what caused the epidemic outbreaks of this insect in certain years. In 1934 and 1935, for instance, the infestation of the aphids in the grove in Rehovoth was normal; it became exceptionally heavy during the spring of 1936, and was very slight during the spring of 1937. What factors lie behind these degrees of infestation? The question of availability of food does not enter into consideration now, since the *Citrus* trees develop new growth every year. The cause must lie in the weather conditions of each year, which are variable. For comparison let us take the two extreme infestations, namely, that of the spring of 1936 and of the spring of 1937. As mentioned above, the infestation of 1936 was noticed as early as November-December of the previous year. This infestation increased gradually without any cessation during January 1936, and towards the end of February of the same year it was heavy. Such was not the case the following year.

The early colonies that appeared in October continued to develop throughout that month and during early November. Towards the end

of this month they disappeared and were not to be found in the grove during December of that year nor during the following month. New small colonies appeared only in February and lasted throughout March but disappeared during the early part of April. Single colonies were seen throughout the summer, the weather having been exceptionally mild during that time. The curve of this infestation, based on the same scale as that of 1936, is presented in Fig. 3.

The chief difference between the two curves is that the curve of 1935–6 rises gradually and continuously during December and January, whereas that of 1936–7 descends noticeably during these two months. This descent is responsible for the low peak during March of that year. The period from February to early April was too brief for the insects to multiply to such an extent as to cause a heavy infestation. In the previous year the species continued to develop uninterruptedly from November to March, and this factor was responsible for the high peak in March. If we compare the temperature of December and January in the two seasons, the cause of such a picture is quite obvious. While the winter of 1935–6 was mild, that of 1936–7 was severe (see Fig. 4). In the latter the daily maximum temperature was, as a rule, below the line of 18° C., while in 1935–6 the maximum temperature was generally above that line. As mentioned in the foregoing, the development of aphids at a temperature below 18° C. is quite slight and of long duration. Reproduction is also more rapid above the line of 18° C. than it is below the line. In addition, the winter of 1936–7 was accompanied by heavy, dry Eastern desert winds, and during the nights the temperature fell to freezing point while the humidity was quite low. The previous winter, on the contrary, was quite mild.

The economic status of the insect

As a rule the aphis causes damage to young groves only because there is always an abundance of new growth in young trees. However, the damage is not very serious since the development of the trees in the summer months is sufficient to make up for the loss suffered during February-March.

More serious damage may result when a heavy attack occurs in a mature grove at the time of blossom. The feeding of the pest on blossom shoots causes the blossom to drop prematurely. Reports of damage of this kind were received particularly in the spring of 1936 from Petah Tikvah, Rehovoth and Ness Ziona.

Blossoming occurs, as a rule, after the peak of the infestation has been attained, i.e. when the aphid population is on the decline. The

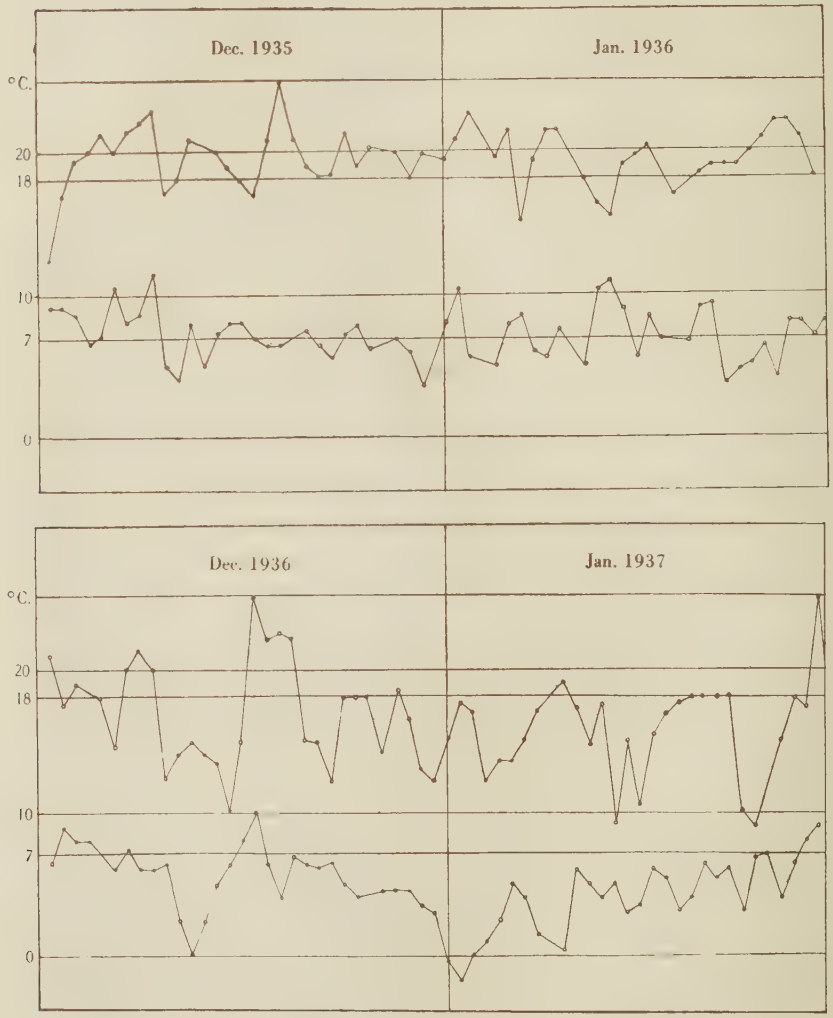

Fig. 4. Daily fluctuations in the temperatures of December and January in 1935–36 and 1936–37.

damage then cannot be considered serious. In 1936, because of the mild winter, spring foliation, as well as blossoming, occurred a few weeks

earlier than the normal time. Fig. 3 shows the difference in these phenomena as they occurred in 1936 and 1937 in Palestine. It is distinctly shown how the blossom period of 1936 coincided with the aphis infestation.

A close survey of such an infestation was made in a grove in Ness Ziona, the following facts being revealed:

(1) Most of the trees attacked were well protected from the south so that the hot, dry desert winds blowing from that direction could not affect the aphis on them as readily as on other unprotected trees.

(2) As a rule, only the northern side of such trees was injured, while the southern side, facing the desert wind, was free from such infestation. Due to the retarding effects of the Khamsen winds, the aphid could continue their damage to a later date than would have been possible otherwise.

Practical suggestions

A mild winter, when the daily maximum temperature during December and January is above the line of 18° C., may encourage the infestation of *Toxoptera aurantii*, which will become most abundant during the month of March.

If measures of control are to be taken it should be done during February, before the leaves have curled. A solution of nicotine sulphate 1 : 800–1 : 1000 proved to be satisfactory against this pest.

Summary

1. The *Citrus* aphis reproduces parthenogenetically continuously throughout the year; sexual reproduction has not been observed.

2. The most favourable temperature for development and reproduction of the aphis is between 22 and 25° C. Between 18 and 22° C. the rate of reproduction and development is also sufficient to bring about heavy infestation.

3. The temperature below 18° C. is unfavourable for considerable increase of the population of this insect. At 7° C. reproduction is barely taking place.

4. At 30–32° C. the mortality of the nymphs is almost 100%. At 34–35° C. reproduction ceases, and at 36° C. adult insects die within a short period.

5. The annual fluctuations of the population of the insect based on field observations are described.

6. Food as a factor in the increase of the population of the aphis is discussed.

7. The effect of the annual fluctuations of the temperature in Palestine upon the population of the aphis is pointed out.

8. The economic status of the insect is discussed.

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(*Received* 1 *October* 1937)

STUDIES OF THE BIOLOGY OF THE DEATH-WATCH BEETLE, *XESTOBIUM RUFOVILLOSUM* DE G.

II. THE HABITS OF THE ADULT WITH SPECIAL REFERENCE TO THE FACTORS AFFECTING OVIPOSITION

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(With 6 Text-figures)

CONTENTS

Introduction

A general account of past work on the death-watch beetle, *Xestobium rufovillosum* De G., was given in the first paper (Fisher, 1937) of this series. The results of part of recent studies at Princes Risborough on the biology of the insect are discussed in the present contribution, which deals particularly with the adult and the effect of temperature and humidity on oviposition.

A serious handicap at the outset of the investigation was lack of insect material, for as Lefroy (1924) also found, beetles could not be obtained in sufficient numbers from timbers from buildings in which damage had been discovered. Moreover, the size of the timbers concerned and the problem of suitable storage space to allow of regular handling and examination rendered unsuitable such a doubtful source of supply of the insects. Abundant material was eventually obtained from decayed parts of willow trees along the banks of streams near Thame, Oxfordshire. Frequent visits were made to these trees, two of which were removed and transplanted at the Laboratory.

In addition to life-history studies carried out in the laboratory, inspections of buildings undertaken at the request of authorities in charge of repairs afforded valuable opportunities of studying the insect in the conditions under which it is often responsible for serious damage to structural timbers.

Pupal period and emergence

In the literature on *Xestobium rufovillosum* there is conflicting evidence as to the time of year at which emergence from the wood takes place and how this is related to the date of completion of the pupal period. For instance, in describing the life history of the insect in its natural habitat out of doors, Munro (1928) states that pupation occurs in spring but the beetles rarely come out of the timber until autumn or even the following spring. Lefroy (1924), forecasting the probable life cycle, indicates that pupae may be found during June and September, and that the beetles emerge in May and June of the following year. Gahan & Laing (1932), and Kimmins (1933–4) state that pupation occurs in late summer and early autumn but that the beetles do not emerge until spring. In view of these differences of opinion, observations have been made in the field, in buildings and on timber removed from buildings and subsequently stored under cover at the Laboratory. In discussing the results of this work it is convenient to deal separately with

the time of pupation and emergence of the insect under natural conditions out of doors, and under artificial conditions in buildings.

A. *Out of doors*

The earliest date at which pupae were found in the open in decayed parts of willow trees was 18 July (1935). During the remainder of this month, in August and occasionally in early September, pupae were found in the trees under observation over a series of years. The pupal period was determined by locating pupating larvae which were removed and kept out of doors and as Table I shows, lasted from 3 to 4 weeks, there being little difference between the sexes. According to Kimmins (1933–4) the period occupies about 3 weeks under "laboratory conditions".

Table I

Duration of pupal stage out of doors, 1935

Sex	Pupal period	Duration (days)	Av. temp. °C.	Av. R.H. %
♂	20–23. vii. to 12. viii.	21–24	17·8	69
♂	20–23. vii. to 12. viii.	21–24	17·8	69
♀	5. viii. to 28. viii.	24	18·4	71
♀	7. viii. to 26. viii.	20	17·8	71
♀	5. viii. to 1. ix.	28	17·8	71

It follows from the above that from August onwards immature beetles are present in infested trees, but observations have shown that they remain inactive throughout the autumn and winter within their pupal cells or in honeycombed wood and normally do not emerge until April and May of the following year. The earliest dates at which emergence was noted were 25 April 1928, and 23 April 1935, but the majority of beetles emerge during May; a few do not appear until June. The following records of the average outside temperatures (in ° C.) at Princes Risborough during these months is of interest for comparison with those in buildings at the time of emergence of the beetles:

	March	April	May	June
1934	5·2	8·4	12·2	15·4
1935	6·7	7·7	10	16·1

The average temperature out of doors at the time of maximum emergence is, therefore, in the neighbourhood of 10–12° C., but emergence probably occurs only on days when the temperature is above the average for the period.

The progress of development of the reproductive organs, described by Fisher (1937), during this period of hibernation is worthy of note.

In the female the rate of development of the ovarioles is slow. For approximately 2 months after pupation no traces of oocytes are usually visible, whilst fat body is abundant. Subsequently, traces of two or three developing oocytes can be distinguished near the base of each ovariole and development proceeds so that by April, at the time of emergence, although not fully mature, two to three well-developed eggs are present in each ovariole; fat body is still present but not abundant.

In the male the progress of development is more striking by reason of the marked changes that occur in the shape and size of the testicular follicles. For a short time following completion of the pupal period the follicles are elongate and cylindrical. This stage of development was noted in beetles collected from trees only during August and early September. By the end of September or early October the follicles have lost their cylindrical appearance, become smaller and pear-shaped as their contents pass into the vasa deferentia and seminal vesicles, which as a result are distended by the seminal fluid. Throughout the remainder of the winter a further diminution in the size of the follicles takes place, until in beetles examined between December and April they appear as minute oval bodies visible only with difficulty. By this time fat body is practically absent and, at emergence, the males are sexually mature.

B. *In buildings*

It has not been possible to procure as full information upon the time of pupation and emergence of *Xestobium* from timber in buildings as from infested trees. Examination of timber shortly after removal from the roofs of churches has, however, shown that in such buildings, unheated or heated only intermittently throughout the winter, pupae may be found at approximately the same time of year as out of doors, viz. August and September. On the other hand, it has been found in the course of experiments in the laboratory that pupation may occur throughout the year, depending upon temperature, humidity and other conditions affecting the rate of development of the larvae. This aspect of the investigation is outside the scope of the present paper, but it is opportune to note the duration of the pupal stage (Table II), when the temperature and humidity at which the development of the earlier stages of the insect (egg and larva) were completed, are considerably higher than those normally prevailing in the open.

From Table II it is apparent that under such conditions the pupal period is about 1 week less than that out of doors, but these conditions are unlikely to occur in buildings unless artificially heated during the

normal time of pupation of the beetle in the late summer. A comparison of the average temperatures prevailing out of doors at this time and in buildings in which death-watch beetle activity was known to occur showed that little difference existed in the two situations. Although the extremes of temperature were greater out of doors, a marked prolongation of the pupal period did not result. It can, therefore, be concluded that the slight difference between the length of the pupal stage out of doors and in buildings has little effect upon the subsequent date of emergence of the adults and the duration of the life cycle as a whole.

Table II

Duration of pupal stage under experimental conditions

Sex	Pupal period	Duration (days)	Av. temp. °C.	Av. R.H. %
♂	19. viii. to 4. ix.	16	23·9	89
♂	19. ix. to 6. x.	18	22·7	90
♀	15. ix. to 2. x.	18	22·7	90

The earliest date at which beetles appeared in a church kept under close observation in 1934 was 7 April. Other records of the occurrence of beetles in buildings have, however, been obtained from mid-March to early June, but the majority of the insects are to be found during the latter part of April and in the beginning of May. The average temperature recorded in the vicinity of infested timber in a building at the commencement of the emergence period was 10° C., which corresponds closely to that out of doors in May, the time of year at which maximum emergence takes place. Dissections of beetles taken from timber from infested buildings during the winter months showed that they also were sexually immature.

When examining timber stored outside for several months after removal from buildings, pupae were obtained on two occasions at times not in accordance with the general habit of the insect as described above. In the first instance, in April and May 1932, six partly coloured pupae, together with numbers of beetles, were cut from alder beams which had been removed from a cottage in June 1931. In April 1933, beetles were obtained but no pupae were then found. In the second case two pupae were taken at the end of January 1933 from deal flooring stored under cover out of doors after removal from a building in August 1931; beetles were again found at the same time as the pupae. A satisfactory explanation of these occurrences of pupae in the early spring has not been found, but is most probably linked up with the previous history of the timber upon which full information was not available, and its effect upon the

duration of the larval stage. In this connexion, mention has already been made of the effect of temperature and other conditions, as shown by experiment, on the duration of the larval stage of *Xestobium*, leading to pupation and emergence of the adults at abnormal times of the year. Moreover, it is to be noted that neither of the timbers concerned is commonly attacked by the death-watch beetle. The occurrence of *Xestobium* pupae in spring is evidently unusual and an exception to the normal life cycle of the insect out of doors or in churches.

These special instances apart, the results of the observations out of doors and in buildings confirm in general those of Gahan & Laing (1925), and of Kimmins (1933–4) that death-watch beetles may be expected to emerge and oviposit during the period mid-March to early June. The condition of the male and female reproductive organs at the time of emergence indicates that the males, which emerge before the females, are sexually mature and capable of proceeding at once to pairing. The females, although not so advanced in development, are rapidly approaching maturity and, as shown later, are ready for copulation. The adults of both sexes do not bore after emergence nor do they feed. Under natural conditions out of doors the sexes occur in approximately equal numbers. For instance, dissection of 167 beetles collected from willow trees in March 1934 showed eighty-six to be males and eighty-one females.

Although emergence is restricted to a definite time of year, normally there is no period at which the insects appear in large numbers. Observations and experiments have shown that the beetles usually emerge a few at a time, and unless looked for carefully, their presence either on the surface of infested trees or in a building can easily be overlooked. On the other hand, in cases of severe infestation when timbers are in a condition especially suitable for attack, a big emergence may take place over a period of several days depending upon temperature conditions. It has been suggested by Lefroy (1924) that true emergence may not always take place, and that in severely damaged timber in a honeycombed condition egg-laying occurs without the parents leaving the timber in which they completed their development. Whilst no definite proof of this has been obtained, support is given to the suggestion by the finding of adults during the normal time of egg-laying within tunnels in oak structural timbers and by the extreme difficulty with which beetles have been collected without cutting up and submitting the timber to a most detailed examination.

HABITS OF ADULTS

Activity

The beetles are normally inactive and out of doors are usually to be found on the underside of loose pieces of bark, in old tunnels or other sheltered places in the vicinity of attacked wood. On sunny, warm days, they have been seen wandering over the surface of attacked trees, tapping frequently; the time of maximum activity is in the late afternoon from 4 to 6 p.m. Kimmins (1933–4) suggests that they are most active during the night, but this is not confirmed by our observations. On the contrary, it appears that activity is governed largely by temperature and by sunlight. For instance, beetles kept in the laboratory were active and moved about slowly at a temperature of 14° C., but their activity greatly increased when the temperature rose to 17–20° C., tapping, pairing and egg-laying being observed.

Tapping

The characteristic tapping produced by the beetle has been the subject of discussion in earlier literature (Allen, 1695; Derham, 1701; Westwood, 1839–40; Altson, 1922; Kimmins, 1933–4) and does not call for special comment. It is sufficient to record that our observations confirm those of the later workers and that the sound is produced by the insect rapping its frons seven or eight times in quick succession on the surface of the wood and is probably a sex call. Accordingly, it is heard only during the months of emergence, prior to and during oviposition. Tapping takes place when the insects are most active and therefore depends upon the same conditions as those which govern their activity. Although both sexes tap, the male does so more frequently; dissection of a batch of insects noted tapping revealed nineteen males and only two females. Tapping may continue after pairing.

Flight

It is questionable whether the beetles ever fly intentionally. They are capable of flight or at least of using their wings to break a fall or to regain their feet when they have fallen on their backs, but there is no evidence to show that their powers of flight are of importance as a means of spread of infestation, e.g. from building to building. On the other hand, the frequency with which beetles have been found on the floors of churches, of which the roofing timbers were known to be attacked, suggests that they might have originated from the roof, and in the absence of any

visible evidence of flight dropped to the floor below, there to congregate in cracks and crevices at the base of pews, rails, etc. They are capable of crawling considerable distances, and in this way may spread infestation within a building.

Pairing

Although pairing was observed at all times of day and evening, both out of doors and in the laboratory, it was recorded most frequently in the late afternoon and was dependent on conditions favouring the activity of the beetles. Copulation was seen when both insects were on the surface of the wood, but it is probable that it can also take place within the tunnels of extensively honeycombed timber. The male mounts upon the back of the female, and when coition has been established gradually falls over backwards and finally rests on its back, with legs and antennae drawn closely into the sides of the body. The female is capable of supporting the male in this position and was seen on occasion to crawl over the surface of the wood with the male thus attached. At laboratory temperature (18–20° C.) the duration of copulation varied from $\frac{1}{2}$ to $1\frac{1}{4}$ hr.

Dissections of females after pairing have shown that the uterus is at first much distended and globular in shape. The contents are white, opaque and of a glutinous consistency. Gradually, however, these disperse and the uterus, passing through an intermediate stage when it becomes pear-shaped, being partly distended and opaque anteriorly but elongate and translucent posteriorly, assumes its normal appearance after 4–6 days, under out-of-door conditions. The presence of a small yellow-brown body in the cavity of the bursa copulatrix has frequently been noted during dissection of females at different stages of development. This substance is absent before and immediately after pairing but is invariably present in fertilized females in which the uterus has regained, or almost regained, its normal appearance, irrespective of whether or not egg-laying has taken place. The origin or significance of this substance has not been determined.

Oviposition

Methods of study

In studying oviposition and the duration of the egg stage, an endeavour has been made to determine the effect of different relative humidities and temperatures with the object of assessing the importance of these factors in relation to the duration of the life cycle of the insect in buildings and out of doors. Numbers of beetles, segregated into individual pairs

whenever possible, were confined in a series of vessels kept at different temperatures, and in which the relative humidity of the atmosphere was controlled by mixtures of sulphuric acid and water (Wilson, 1921). In addition, beetles were placed in other vessels out of doors and meteorological records kept. The material offered to the insects for oviposition was oak sapwood and willow, previously decayed by *Phellinus cryptarum* Karst and *Ganoderma applanatum* (Pers.) Pat., respectively, each fungus producing a white rot. Sound oak sapwood and willow were also used as controls. The choice of decayed timber was the outcome of experiments which showed that wood in such a condition was specially suitable for the development of the larvae of *Xestobium*. The samples were placed in the vessels to condition to an equilibrium moisture content in accordance with the relative humidity of the atmosphere in each.

In view of the habit of the insect of over-wintering in the adult stage, and in order to be certain that the beetles used had not paired before collection, it was usually necessary to remove them from infested timber or trees during the winter months and keep them under close observation in the different environmental conditions concerned. The absence of external sex characters was a serious handicap in this work.

Mode and location of egg-laying

Oviposition was never observed, nor have eggs been found in timber under natural conditions in trees and in buildings. On the few occasions when egg-laying was observed in the laboratory the female beetle moved slowly over the surface of the wood exploring all pits and crevices, first with her antennae and then, turning round, with the palps at the tip of the ovipositor which was protruding slightly beyond the everted last abdominal tergite. On locating a suitable crevice the ovipositor was extruded and inserted therein: about 5–7 sec. elapsed before contraction of the abdomen and sudden progressive dilatation of the ovipositor showed that an egg was being deposited. Several such crevices were carefully marked and subsequent examination showed an egg in each. In one case under observation, a female seen to be ovipositing at 11.15 a.m., when the laboratory temperature was 20° C., continued egg-laying at intervals throughout the day.

In order to determine whether oviposition is more frequent during day or night, counts were made of the numbers of eggs laid by individual beetles throughout their life, between 10 a.m. and 10 p.m. and between 10 p.m. and 10 a.m., in the laboratory and out of doors, during June and July 1936. The results showed that although occasionally eggs were laid

between 10 p.m. and 10 a.m. the following day, by far the greater number were laid during the day and early evening. In view of the observed activity of beetles in the late afternoon it is probable, therefore, that egg-laying takes place most frequently at that time.

In experiments in the laboratory, eggs were inserted singly, in pairs and sometimes in small groups, in cracks or crevices, on roughened surfaces, among broken fibres of wood, preferably on the transverse surface. They were also noted within the vessels of oak, and in all situations were inserted as deeply as possible so that it was frequently difficult to detect their presence. Eggs laid under loose chips were invisible unless these were raised or pulled to one side. Instances were also recorded of the presence of eggs in the frass of old tunnels. It was not uncommon to find groups of eggs varying in number from five to six up to as many as 150 on the exposed surfaces of samples, more frequently between adjacent specimens, or on the under surface between the sample and floor of the vessel. When in groups, each egg was attached to, or at least in contact with, its neighbour and adhered to the substratum probably by a cement on the chorion which set soon after the egg was laid.

From these observations it seems probable that, in timber in buildings, beetles oviposit in a great variety of situations and conceal their eggs by whatever means are offered, according to the condition of the wood, by inserting them in cracks, crevices and open joints at the junction of individual timbers, or hiding them among old larval frass. There is no evidence to suggest that the choice of site for oviposition can be correlated with the suitability of the surrounding timber for the young larvae to commence boring. On the contrary, beetles offered a choice of decayed and sound oak sapwood, each provided with cracks and crevices, did not show a preference for ovipositing in the decayed specimens. The ability of the larvae, on hatching, to crawl over the surface of the timber, presumably in search of a suitable place to commence boring, compensates for any handicap resulting from oviposition having taken place in a situation unsuitable for their future development.

Records of egg-laying and duration of egg stage

In the experimental work on oviposition and the duration of the egg stage, accurate records were frequently difficult to obtain on account of the position of the eggs. This source of experimental error, must, therefore, be taken into account in considering the results obtained. It is convenient to deal separately with the observations and experiments

out of doors and under controlled conditions of temperature and humidity.

A. *Out of doors*

Records of egg-laying were obtained by keeping beetles, including segregated pairs, under observation in muslin-covered glass dishes. In some instances desiccators were used in which the relative humidity was maintained at 86%. A summary of the results is given in Table III.

Table III

Egg-laying records out of doors

Exp. started	No. of beetles ♂	No. of beetles ♀	Date paired	Date 1st eggs laid	Av. duration of egg-laying (days)	Av. eggs per ♀
30. i. 33*	5	4	—	29. iv. 33	46	44
31. i. 33*	2	5	—	27. iv. 33	65	60
6. ii. 33*	3	4	—	29. iv. 33	63	50
4. iv. 34	5	2	—	15. v. 34	18	77
8. v. 34	1	1	8. v. 34	17. v. 34	16	75
8. v. 34	1	1	8. v. 34	15. v. 34	12	54
8. v. 34	1	1	8. v. 34	21. v. 34	16	59
23. iv. 35†	1	1	23. iv. 35	28–30. v. 35	24	140
25. iv. 35†	1	1	25. iv. 35	28–29. v. 35	25	39
25. iv. 35†	1	1	25. iv. 35	31. v. 35	36	108

* At 86% relative humidity.
† Found pairing on surface of tree but may have paired previously.

The interval of 8–13 days between pairing and egg-laying, recorded in Table III, agrees comparatively closely with that of 5–11 days given by Kimmins (1933–4), who does not, however, state whether his figures refer to insects kept out of doors. The observations in general suggest that this period is governed by a number of factors amongst which the most important are the state of development of the ovaries at the time of pairing and temperature conditions during subsequent weeks. For instance, other records show as many as 36 days to elapse between pairing and egg-laying, and in this connexion it is significant that in two experiments started in 1934 at an interval of 1 month, egg-laying commenced in both at approximately the same time (May) and continued for 2–3 weeks, during which the number of eggs laid per female varied from fifty-four to seventy-seven. It is of interest to compare with these results the egg-laying records obtained in 1933 from the three experiments in which the relative humidity was kept at 86%, rather higher than that (approximately 70%) of the open air at this time of year. Egg-laying began about the same time in each, but earlier than in 1934 and continued over a much longer period, 6–9 weeks, but the average number of eggs

per female was only forty to sixty. Meteorological data were not available at Princes Risborough in 1933, but it is significant that, according to the monthly returns of the Meteorological Office, temperatures in general from March to May of that year were considerably higher than those for the corresponding period in 1934. The date of commencement and the duration of egg-laying are, therefore, probably correlated with temperature conditions. Further evidence in support of this has been obtained from experiments described later.

It is also evident from Table III that there is considerable variation in the number of eggs laid per female. For instance, in the records of oviposition of individual pairs of beetles, the number of eggs per female varied from as few as thirty-nine to as many as 201. The usual number of eggs per female was forty to sixty. It should be explained that, as a result of a difference in fecundity of individual beetles, as affected, for instance, by a variation in the number of ovarioles per ovary (Fisher, 1937), the potential egg-laying capacity of different females will vary considerably. Moreover, as is shown later, conditions of temperature and humidity also play an important part in this respect.

When it was possible to determine accurately the length of life of individual beetles, observations showed that the females may live up to 10 weeks after pairing; the males are shorter lived, surviving 8–9 weeks after pairing. In no case under out-of-door conditions were beetles of this generation found alive after the beginning of July. It will be recalled that pupation is completed in July and August, 7–8 months before the beetles emerge from the wood for pairing and egg-laying. The total length of adult life of the death-watch beetle is, therefore, from 10 to 11 months, during only three of which, April-June, egg-laying takes place.

Incubation period of the egg. The duration of the egg stage was determined by examination of the samples from which egg-laying records were obtained. The mean duration of the stage ($\bar{x}$), given in Table IV was calculated as

$$\bar{x} = \frac{\Sigma n_1 r_1}{n},$$

where n_1 is the number of eggs hatching after a recorded period, r_1 the duration of this period in days, and n the total number of eggs hatching.

A comparison of the records in Table IV, in which the egg period varies from 3 to 5 weeks, with the mean temperature at Princes Risborough from May to July in 1934 and 1935, suggests that this variation is due to the differences in temperature prevailing throughout the egg stage

Table IV

Duration of egg stage out of doors

Date of oviposition	Eggs	Mean duration of stage (days)	Av. temp. ° C.
1934: 15. v. to 1. vi.	297	35·7	14·5
1935: 28. v. to 31. v.	136	35·4	15·6
15. vi. to 21. vi.	114	25·2	18·3
23. vi. to 5. vii.	8	21·9	18·7

according to the date of oviposition. For instance, the average temperature out of doors during the development of the 136 eggs laid in the end of May was 14·5° C. as compared with 18·3° C. during the incubation period of the 114 eggs laid in the end of June and beginning of July. The effect of constant temperature and humidity on the duration of the egg stage is discussed later, but it is evident from the above records that out of doors this period is normally about 5 weeks, since the majority of the eggs are laid before the end of May.

Viability of eggs. The viability of the eggs as determined by the percentage hatching after allowance has been made for those accidentally destroyed or lost is given in Table V.

Table V

Viability of eggs out of doors

Year	No. of eggs	% hatched
1934	315	97·8
1935	339	93·3

Average R.H. during egg period = 70% (approx.). Range of average daily temperature during egg period = 14–21° C.

The average survival of *Xestobium* eggs out of doors is therefore about 95%.

B. *Under controlled conditions of temperature and humidity.*

In order to obtain further information upon the effect of temperature and humidity on oviposition, a series of experiments was carried out at relative humidities between 23 and 95% and at temperatures of 15, 20, 25 and 30° C. Some difficulty was experienced in maintaining a constant temperature of 15° C., fluctuations of ±3–4° C. occurring, but the mean temperature was approximately correct. The majority of the experiments were started at the same time during April and May, the normal period of emergence and egg-laying, and are therefore supplementary to those described above under out-of-door conditions. As far as could be determined by size, in the absence of secondary sex characters, four pairs

of beetles were used in each experiment; the sex of all individuals was ascertained by dissection at the conclusion of the work. In discussing the results which are summarized in the following tables, it should be noted that the records were obtained from observations on groups of insects, the individuals among which varied in egg-laying capacity, duration of life, etc. Such variations in themselves may mask the effect, if any, which conditions of temperature and humidity exert, but it is believed that sufficient data are available to enable general conclusions to be drawn.

Table VI

Effect of temperature and humidity on interval between emergence and oviposition

Exp. started	R.H. %	Interval (days) between start of exp. and date of first egg-laying			
		15° C.	20° C.	25° C.	30° C.
4–5. iv. 34	23	18–21	7–8	7	9
4–5. iv. 34	41	18–21	9	6–7	6
4–5. iv. 34	53	23–26	7–8	8	6
4–5. iv. 34	75	18–21	9–10	8	7
25. i. 33*	86	—	—	12–13	—
1. ii. 33*		—	—	13	—
6. ii. 33*		—	15	—	—
21. viii. 33*		—	—	80–90	—
29. ix. 33*		—	126	—	—
4–6. iv. 34	95	14	10	8	6

* Beetles used removed from wood prior to or during hibernation.

It is evident that, humidity apart, temperature is an important factor in determining the date at which egg-laying commences. Thus, the records show in general that during the normal egg-laying period (May) an increase in temperature results in earlier oviposition. The effect of a difference in temperature of 5° C. at 15 and 20° C. is most marked, but above 20° C. is less pronounced. It is also apparent that a temperature considerably above that which normally prevails in the open can result in egg-laying taking place during the winter months. Under such circumstances the date of first egg-laying is also governed by the state of development of the beetle at the time of exposure to the increase in temperature. For instance, when beetles taken from pupal chambers in trees in August and September, i.e. about 1 month after completion of the pupal period, and therefore before hibernation, were exposed to temperatures of 25 or 20° C. and a relative humidity of 86 %, the earliest dates of egg-laying were during November and the following January respectively.

Relative humidity does not appear to be an important factor in determining the time of oviposition.

Duration of egg-laying and number of eggs laid per female. The effect of temperature and humidity on the duration of egg-laying and on the number of eggs per female is shown in Table VII.

Table VII

R.H. %	Duration of egg-laying (days)				Av. no. of eggs per ♀			
	15° C.	20° C.	25° C.	30° C.	15° C.	20° C.	25° C.	30° C.
23	23–26	17–18	8	9	30	60	20	7
41	23–26	15	15–16	10	34	54	23	24
53	18–21	18–19	9	15	31	45	45	24
75	34–37	24–25	21	14	52	37	45	45
95	31	41	17	24	61	79	115	50

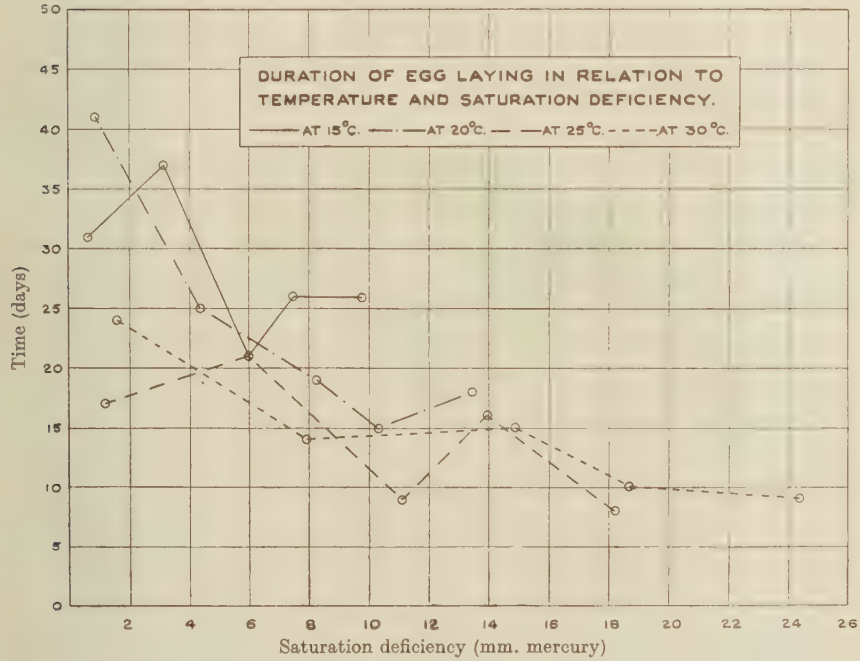

Fig. 1

These records show that between 15 and 30° C. there is a tendency for the egg-laying period to be prolonged as the relative humidity rises, accompanied, at the higher humidities, by an increase in the number of eggs per female. An increase in temperature tends to shorten the egg-

laying period, and up to 20° C. to increase the number of eggs per female. There are indications that at a higher temperature the number of eggs per female decreases when the humidity is low. If, however, the humidity increases, the oviposition records also increase up to 20–25° C., thereafter falling off. These observations suggest therefore that temperature and humidity together play an important part in governing oviposition and

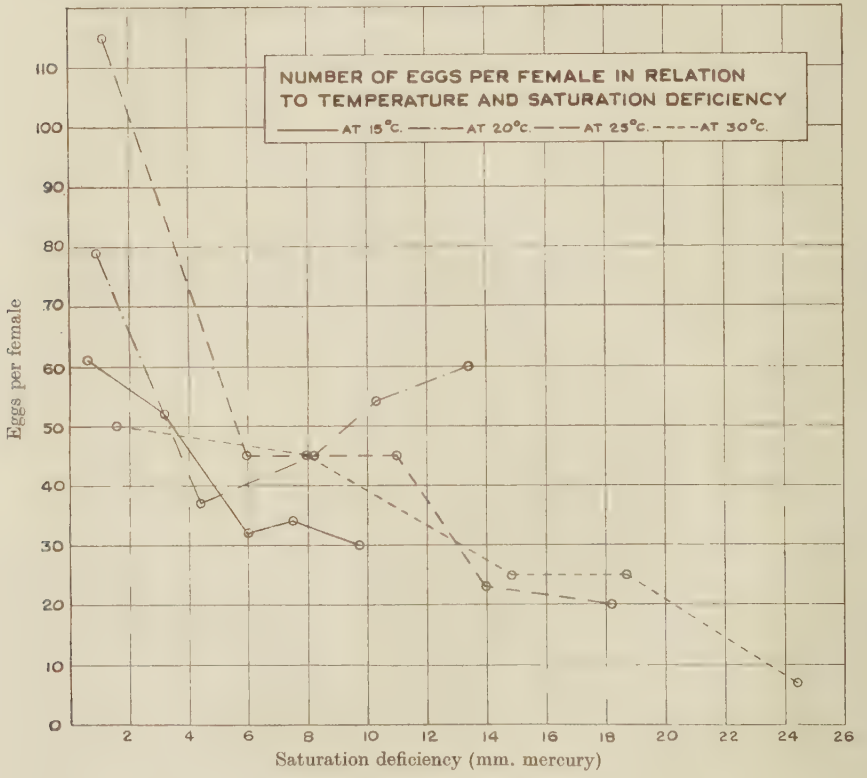

Fig. 2

its duration, the lower temperatures accompanied by high humidities being more favourable. As Buxton (1931) has suggested, such an effect can best be shown by comparing the results with the saturation deficiencies corresponding to the different temperatures and humidities used. If this be done (Figs. 1 and 2) it becomes evident that, in general, dry conditions curtail the duration of egg-laying and decrease the number of eggs per female. Furthermore, the effect of various temperatures, as apparent in

Table VII, is entirely explained by the different saturation deficiencies which they cause.

Duration of life of adults after emergence. Records were kept of the length of life of the beetles used in the oviposition experiments, and the results, summarized in Table VIII, refer to this period as calculated from the commencement of these experiments in April at the normal time of emergence and the start of egg-laying.

Table VIII

Average duration of life of adults (days)

R.H.	15° C.		20° C.		25° C.		30° C.	
%	♂	♀	♂	♀	♂	♀	♂	♀
23	36	46	19	24	17	19	14	19
41	44	52	21	25	14	21	14	16
53	42	56	22	28	20	22	16	21
75	52	56	24	34	19	29	15	23
95	36	63	27	49	22	35	15	34

These figures show that the length of adult life again varies according to conditions of temperature and humidity. Thus, at any one humidity, an increase in temperature shortens the period, but at a given temperature a rise in humidity has the opposite effect, tending to prolong adult life. At the lower temperatures, 15–20° C., approximating to those out of doors, the duration of life of the female varied, according to conditions of humidity, from 7 to 9 weeks approximately; under similar conditions the males lived from 5 to 7 weeks. At a temperature of 30° C., on the other hand, the duration of life of the female was only 3 to 5 weeks, and that of the male about 2 weeks. These conclusions are confirmed by examination of the curves (Fig. 3) showing the relation between the duration of life of the female and the saturation deficiencies corresponding to the temperatures and relative humidities cited in the above table. As the saturation deficiency decreases adult life is prolonged. Furthermore, the effect of temperature is not entirely included in the changes in saturation deficiencies as shown in these curves, since an increase in temperature from 15 to 20° C. shortens the length of life of the female but a rise from 20 to 30° C. has little further effect.

These observations support the conclusions reached from the out-of-door experiments, and show that the active life of the adults is confined to a comparatively short period in late spring and early summer. Thereafter, they die off, none surviving later than early July.

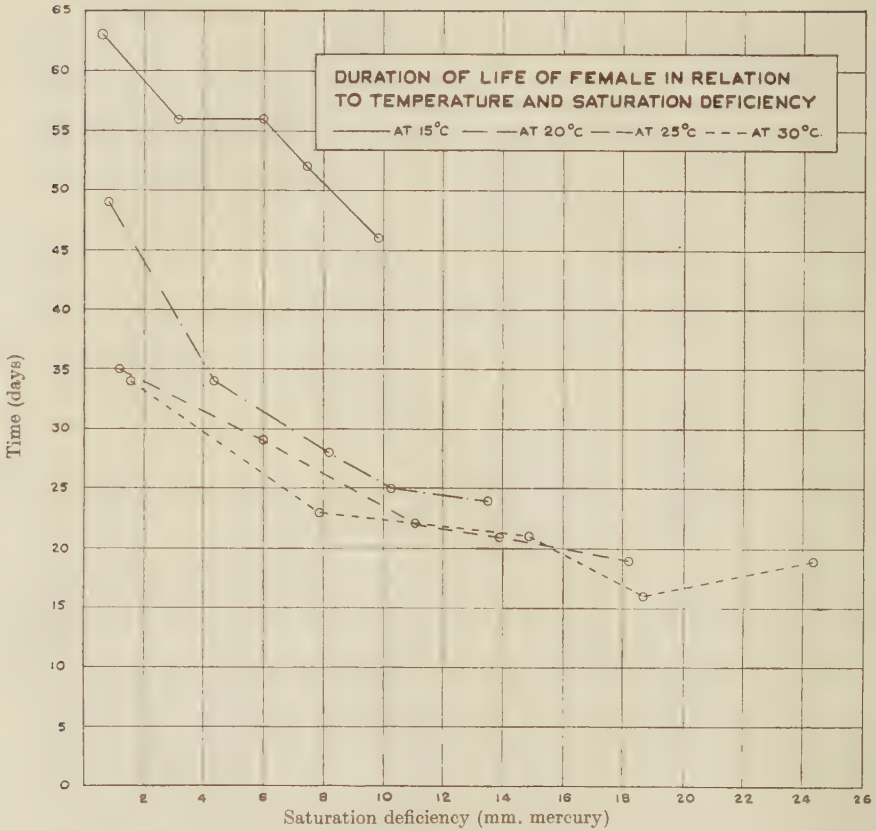

Fig. 3

Incubation period of the egg. The effect of temperature and humidity on the duration of the egg stage is shown in Table IX and represented graphically in Fig. 4.

Table IX

Duration of egg stage (*days*)

Temp. °C.	R.H. % 23	41	53	75	86	95
15	No hatching	49·7	41·3	45·6	—	44·9
	(*91*)*	(*132*)	(*78*)	(*189*)		(*80*)
20	No hatching	23·6	21·3	20·01	20·7	21·1
	(*119*)	(*123*)	(*82*)	(*73*)	(*83*)	(*399*)
25	No hatching	15·3	15·7	14·2	15·7	12·5
	(*118*)	(*41*)	(*18*)	(*133*)	(*180*)	(*96*)
30			No hatching			
	(*29*)	(*118*)	(*116*)	(*178*)	(*22*)	(*248*)

* Figures in italics refer to numbers of eggs.

At each of the temperatures stated, changes in relative humidity above 41 % have little or no effect on the rate of development of the egg. At 23 % on the other hand, at all the temperatures quoted, hatching did not take place. The lowest humidity at which egg development can proceed was not determined, but lies between 23 and 41 % within the limits of the above temperatures. If, however, the duration of the egg stage is plotted (Fig. 4) against the saturation deficiencies corresponding

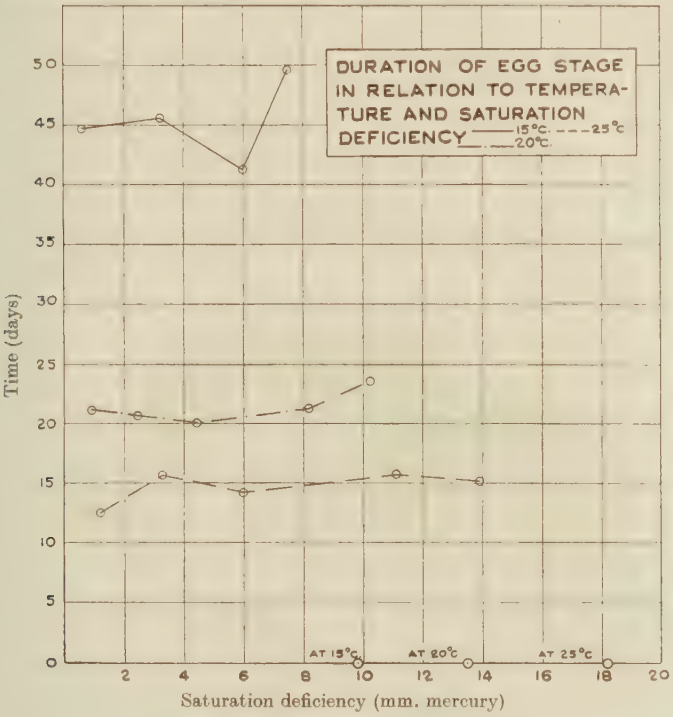

Fig. 4

to the different relative humidities at each temperature, it is evident that the degree of dryness which the eggs can tolerate varies with the temperature. Within the range of effective temperatures, a rise brings about an increase in the degree of dryness that can be tolerated by the eggs without affecting the duration of the stage, thus:

Temp. °C.	Max. saturation deficiencies for completion of egg state (mm. of mercury)
15	Between 7·5 and 9·5
20	„ 10·3 „ 13·5
25	„ 13·98 „ 18·2

Furthermore, it is evident (Fig. 4) that although dryness has only a limiting effect on the duration of the egg stage, temperature, as the results of observations out of doors suggest, has a progressive effect. Whilst the minimum fatal temperature has not been determined, it lies

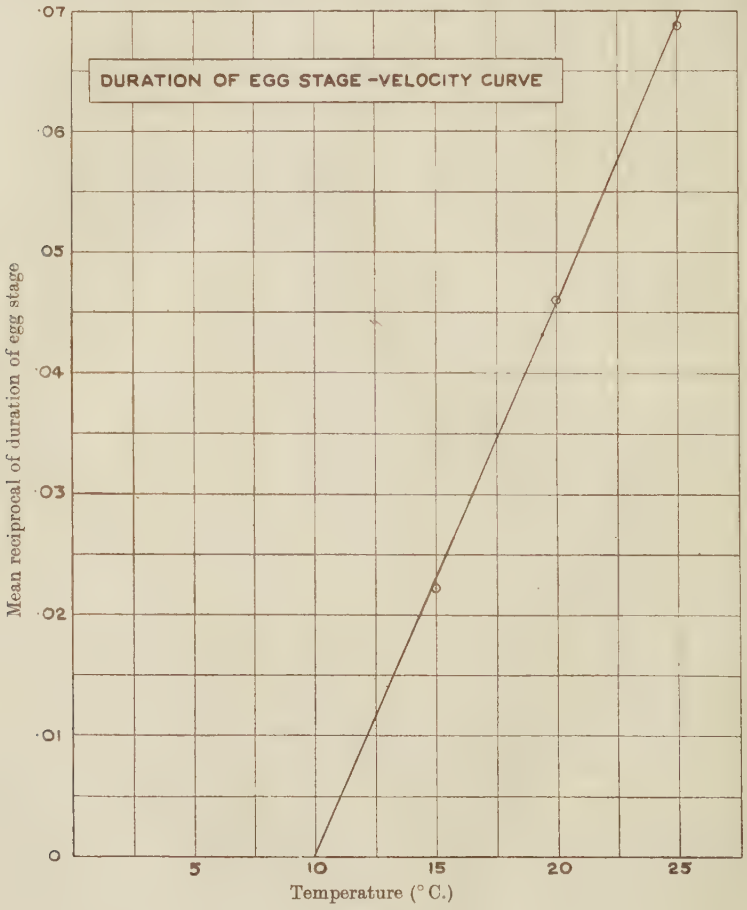

Fig. 5

between 25° C., at which the egg period lasted 2 weeks, and 30° C. at which hatching did not take place. Moreover, it seems probable that 25° C. is approaching the optimum temperature for the duration of the egg stage, since under such conditions the egg hatches in a shorter time and can tolerate greater dryness than at lower temperatures. The

separation of the curves at 15 and 20° C., and at 20 and 25° C. supports this suggestion. At the other end of the temperature range under investigation, the mean duration of the stage was approximately 7 weeks at 15° C. A few eggs kept at 10° C. had not hatched after 8 weeks, but when subsequently exposed to a temperature of 20° C. completed their development.

These observations suggest that the threshold of development of *Xestobium* eggs lies between 10 and 15° C. The theoretical threshold as shown in the velocity curve (Fig. 5) obtained by plotting the mean reciprocals of the duration of the egg stage against temperature is in the neighbourhood of 10° C. It is significant that this temperature approximates to that which normally prevails out of doors at the beginning of the emergence period of the beetles at the end of April and during May.

Viability of eggs. The records of viability obtained in the oviposition experiments under controlled conditions in the laboratory are summarized in Table X.

Table X

Viability of eggs

R.H. %	15° C.	20° C.	25° C.	30° C.
23	0	0	0	0
41	85·2	61·3	73·2	0
53	91·3	95·9	87·4	0
75	97·0	92·8	76·8	0
95	86·1	96·4	90·5	0

Very low humidities are unsuitable for the development of the egg; high humidities, on the other hand, are favourable but small changes in humidity at the upper end of the scale do not greatly affect the survival rate. The above figures also show the effect of temperature. Thus, at 25° C., the percentage survival is in general less than at lower temperatures, whilst at 30° C. the lethal temperature, irrespective of humidity conditions, has been passed. If the percentage survival of the eggs is plotted against saturation deficiency (Fig. 6), it is evident that very dry conditions are again unfavourable. Furthermore, at any of the given temperatures, an increase in saturation deficiency has little effect upon viability until a limiting value is reached when viability falls rapidly. Temperature is again seen to have an effect, not only in that the lower temperatures are slightly more favourable for hatching than the higher, but also as a study of the egg period has already shown (Table IX; Fig. 4) that the higher the temperature the greater the degree of dryness tolerated by the egg.

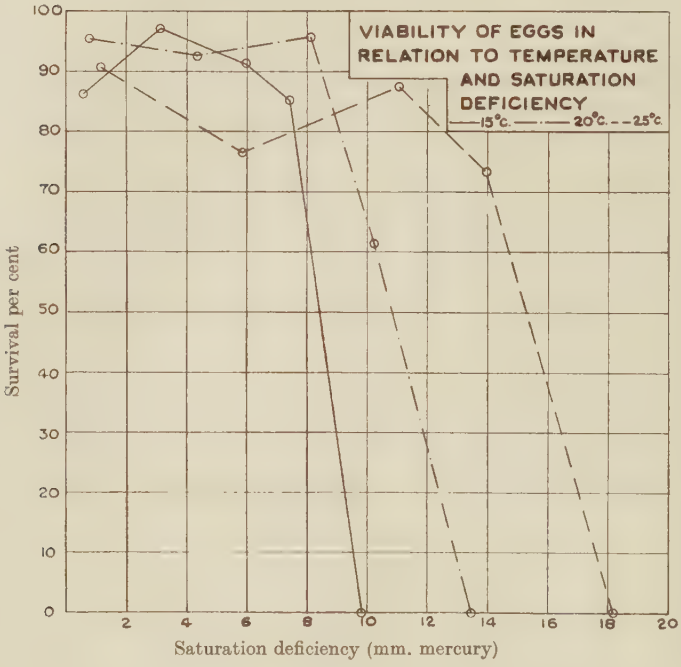

Fig. 6

Discussion

The effect of the different conditions of temperature, humidity and their related saturation deficiencies, on oviposition and life of the adult are summarized in Table XI.

So far as the rate of increase of *Xestobium* is dependent upon conditions favourable for oviposition and the hatching of the egg, the ultimate effect of temperature and humidity can best be determined by assuming that the most favourable conditions are those which allow the maximum number of viable eggs to be laid and hatched in the shortest time. This effect can conveniently be expressed as an "index of suitability" (I) for oviposition and hatching, and can be calculated in terms of the number of eggs laid (N), viability (V), duration of egg laying (L) and the egg period (T) for each combination of temperature and humidity according to the formula

$$I = \frac{NV}{L+T}.$$

Table XI

	Temperature 15–30° C.	R.H. 23–95 %	Sat. def. (dryness) (0·64–24·4 mm. mercury)
Duration of egg-laying (*L*)	Curtailed by increase in temperature, especially at low R.H. %	Prolonged by rise in humidity	Prolonged by decrease in dryness
Number of eggs per female (*N*)	Increased by rise in temperature up to 20° C. (approx.); thereafter decreased	Slight increase at high humidities	Increased by decrease in dryness. No temperature effect other than that represented by saturation deficiency
Duration of life of adults	Curtailed by rise in temperature	Prolonged by rise in humidity	Prolonged by decrease in dryness; additional temperature effect pronounced between 15 and 20° C.
Viability of eggs (*V*)	Decreased at high temperatures	Increased by rise in humidity	Little effect at low saturation deficiency; high saturation deficiency unfavourable, depending on temperature
Duration of egg stage (*T*)	Curtailed by rise in temperature. No hatching at 30° C.	Not affected by humidities above 41%. No hatching at 23%	Limiting effect only, varying with temperature

The most favourable conditions are those which yield the highest index. It is obvious that the numerical value of these indices would be altered if the whole life cycle were taken into consideration. They do however provide a means of comparing the effect of the different conditions on the rate of increase of the insect, during the stages under discussion.

Table XII

Indices of suitability for oviposition, etc., in relation to temperature and relative humidity

Temp. ° C.	R.H. % 23	41	53	75	95
15	0	0·38	0·47	0·61	0·69
20	0	0·85	1·04	0·76	1·27
25	0	0·54	1·57	0·99	3·6
30	0	0	0	0	0

The figures in Table XII show, in general, that a rise in temperature at relative humidities of 41 % and above, to an undetermined maximum between 25 and 30° C., favours an increase in the rate of reproduction of the insect. In addition, there is a general indication that at any one temperature an increase in humidity is also favourable. The optimum conditions appear to be a temperature near 25° C. combined with a high humidity.

By grouping the indices of suitability according to the saturation deficiencies at which they were determined (Table XIII), it is apparent that dryness is unfavourable and that the optimum conditions for egg-laying and hatching must include a low saturation deficiency.

Table XIII

Indices of suitability for oviposition, etc., in relation to saturation deficiency

Temp. ° C.	Saturation deficiency (mm. of mercury)				
	0–4	4–8	8–12	12–16	16 and above
15	0·65	0·43	0	—	—
20	1·27	0·76	0·95	0	—
25	3·60	0·99	1·57	0·54	0
30	0	0	0	0	—

Whilst these figures show that within any one range of saturation deficiency, an increase in temperature to a maximum between 25 and 30° C. is favourable, they also demonstrate the increasing tolerance of dryness associated with a rise in temperature, to which attention has already been directed. Such a conclusion is in agreement with Buxton's (1931) suggestion that the body temperature is a factor controlling the activity or development of an insect. On this supposition the two variables, air temperature and air dryness, may be combined into one effect on body temperature. The tolerance of an increasingly dry atmosphere, as the temperature rises, is explained by the fact that evaporation causes lowering of the body temperature. Thus, increasing the dryness of warm air would assist the insect to accommodate its body temperature to unfavourable air temperatures.

The general conclusions arising out of this study of the factors affecting the oviposition of the death-watch beetle are of importance in relation to its occurrence and spread in buildings. For instance, warm dry conditions are less favourable for the spread of infestation during egg-laying than warm moist conditions which may be localized in structural timbers as the result of a leaking roof or imperfectly watertight gutters. In this connexion, central heating may produce conditions suitable for egg-laying and hatching, in roofing or other timbers, by causing condensation of moisture unless adequate ventilation is provided. Furthermore, these findings offer a possible explanation of the slow progress or even cessation of attack which is often a feature of infestation of timber in buildings by the death-watch beetle. It is possible that high temperatures, associated with fluctuations in humidity, may occur in confined spaces in the roof of buildings and coincide with the period of

emergence and egg-laying, resulting in low egg production and a high egg mortality. Moreover, it is interesting to recall that of the Anobiidae in this country, *Xestobium* emerges in its natural habitat out of doors earlier than most other members of this family and is therefore apparently a species which for egg-laying does not like the higher temperatures prevailing later in the year. Consequently, although temperatures above those occurring in the open may favour oviposition, it is conceivable that as a result the rate of increase of the beetle may ultimately be decreased. In this connexion, Park (1935) has shown that the fecundity of *Tribolium confusum* is markedly affected by exposing the larval stages to a temperature of 39° C. for 5 hr.; the fertility of the beetle is however not affected. In the course of investigations now in progress, on the duration of the life cycle of the death-watch beetle, some evidence has been obtained which suggests that rearing this insect at temperatures above those occurring in its natural habitat may have a similar effect on its rate of reproduction in later generations. This also may be an explanation of the gradual cessation of attack in buildings.

SUMMARY

1. This paper, the second in a short series on biological studies of the death-watch beetle, *Xestobium rufovillosum* De G., is concerned with the life of the adult, egg-laying and hatching.

2. Times of pupation and emergence in the natural habitat of the insect in decayed parts of willow, and in timbers in buildings are discussed and compared.

3. The rate of development of the reproductive organs after pupation and until egg-laying starts, is described.

4. The habits of the adults, activity, tapping, flight and mode of pairing are commented upon.

5. The major part of the paper deals with oviposition which has been studied out of doors and under controlled temperature and humidity in the laboratory.

6. Out of doors forty to eighty eggs per female are laid during May and June over a period of 6–9 weeks. The egg stage lasts from 3 to 5 weeks, according to temperature conditions.

7. In the laboratory, oviposition and the hatching of the egg were studied at 15, 20, 25 and 30° C. and relative humidities of 23, 41, 53, 75, 86 and 95%. The results are discussed in relation to the effect of temperature, relative humidity and saturation deficiency.

8. "Indices of suitability" for various combinations of temperature

and humidity in relation to egg-laying and hatching are calculated from the experimental data.

9. It is shown that an increase in temperature to a maximum between 25 and 30° C. is favourable but that the optimum conditions must include a low saturation deficiency. The data demonstrate an increasing tolerance, by the insect, of dryness associated with a rise in temperature.

10. The application of these findings to the occurrence and spread of *Xestobium* in buildings is briefly discussed.

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ON THE BIONOMICS AND STRUCTURE OF SOME DIPTEROUS LARVAE INFESTING CEREALS AND GRASSES

III. *GEOMYZA* (*BALIOPTERA*) *TRIPUNCTATA* FALL.

By I. THOMAS

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(With 10 Text-figures)

CONTENTS

I. Introduction

The imago of *Geomyza tripunctata* Fall. closely resembles that of *G. combinata* L., and without microscopical preparations it is very difficult to tell the two species apart. According to Balachowsky & Mesnil (1935), however, this can easily be done by a microscopical examination of the male claspers.

Czerny in Linder's *Die Fliegen der Palœarktischen Region* (1928) describes the adults of *G. tripunctata* and *G. combinata*, but it is probable

that Czerny's *G. tripunctata* includes both *G. tripunctata* and *G. combinata*. The author has examined a number of geomyzids in the Cambridge museum which had been classified according to Czerny; the specimens labelled *G. tripunctata* were all that species, and the specimens labelled *G. combinata* were all *G. Balachowskyi* Mesnil. No *G. combinata* were found. Of the specimens bred during the course of this investigation by far the greatest number have been *G. tripunctata* (at first these were thought to include both *G. tripunctata* and *G. combinata*). A few *G. Balachowskyi* have been found but no *G. combinata*.

Since much confusion has existed in the identification of these species, it is possible that the description of the third-instar larva given by Frew (1923) as that of *G. combinata* is really *G. tripunctata*; he records the larva from wheat, barley, *Agropyrum repens*, *Festuca elatior*, *Lolium perenne*, *Holcus lanatus* and *Agrostis alba*. Miles (1913) has also recorded the damage done to *Lolium perenne* by the larva of *Geomyza tripunctata*. Kreiter (1928) has given a short account of the biology of this species and states that there is one generation per annum, the adults appearing during the first days in July. He figures the third-instar larva with only eight projections to the anterior spiracle. Balachowsky & Mesnil (1935) have lately given a short description of the larva of *G. tripunctata*, stating that it often attacks grasses of the genus *Lolium* to a very serious extent. In France there are two generations per annum, the first adults appearing in April.

II. Bionomics

(*a*) *Field observations*

In the field, adults of *Geomyza tripunctata* may be caught as early as the second week in March and as late as the last week in November. They can be swept from almost all grasses, in mid-field, near hedgerows or along roadsides. They are exceedingly active in warm weather, making short flying excursions from clump to clump of grass. They do not become abundant until mid-April. From the end of April until the end of June they become increasingly difficult to find, but from July onwards there is a second increase and flies continue to emerge until October.

Third-instar larvae may be found throughout the winter in *Lolium italicum*, *L. perenne*, *Dactylis glomerata*, *Poa trivialis*, *P. annua* and wheat. In the areas where these observations were made (Lancashire and East Anglia) tillers with their central shoot withered were very common in clumps of *Lolium perenne*. At the base of these shoots were generally to be found larvae of *Geomyza tripunctata* or *Oscinella frit*. Generally the

percentage of the latter was greater than the former but not infrequently clumps of *Lolium perenne* were examined which contained a larger number of the larvae of *Geomyza tripunctata* than *Oscinella frit*. Generally only about 4–5 % of the tillers were found to be attacked, but in one clump of *Lolium perenne* from a roadside, 16 % of the tillers were killed. Only occasionally were larvae found in the other grasses mentioned above or in wheat.

First- and second-instar larvae were more abundant during May and from August to October than during any other periods of the year. Occasional first-instar larvae were found as late as November and a few second-instar larvae were found in January. Both species overwinter mainly as third-instar larvae and pupate during February and March, unhatched puparia being present throughout the spring and summer. Third-instar larvae, however, occur during any period of the year. From the above observations and from breeding experiments in the laboratory it is concluded that this species probably has two generations per annum.

(*b*) *Laboratory observations*

Experiments were designed to determine the egg-laying habits, the larval host plants and the comparative infestation of various grasses and cereals. Seeds were set in pots in a greenhouse, the cereals and grasses used being wheat, barley, oats, *Lolium perenne*, *L. italicum*, *Dactylis glomerata*, *Poa trivialis*, *Cynosurus cristatus* and *Festuca rubra*. Pots were also sown with mixtures of these seeds. From 5 to 12 days after the plants had appeared above the ground, hurricane-lamp cages containing two male and two female flies were placed over as many pots as possible. (The smallest number of pots of any one cereal or grass over which flies were caged was five.)

Oviposition. Eggs were always laid in the stems of the host plants, generally only one egg per plant. Occasionally, however, two or even three eggs were laid in close proximity to each other. The majority of the eggs were laid at, or just below, soil level, a few higher up the stems, but they were never found above a height of 2 in. When the sheath of the host plant was loose or broken, eggs were always found behind it so that they could not be seen without removing the sheath.

Hatching. In a few instances when hatching was observed the young larva crawled upwards until it reached the base of the leaf blade, when it bored downwards either between the outer sheaths or between a leaf sheath and the central shoot. Examination of these plants showed that the central shoot had been bored into about 2 in. from its base where the

larva had eaten out two or three spiral channels until it arrived at the base of the shoot. It is unlikely that all the newly hatched larvae penetrate to the central shoot in the above manner, for in some instances there was evidence of a more direct penetration.

At the base of the shoot the young larva continued to feed until the central shoot was severed. The damage became more noticeable after about 2 days when the shoot died and turned yellow. After 5–8 days the first instar moulted and the second instar fed for 2-4 days when ecdysis again occurred. In the third instar the larvae fed for 9–15 days. Pupation took place inside the shoot; the pupal period varied from 17 to 30 days.

Emergence from the pupal case is effected by means of the *ptilinium*. This structure is seen to be pushed out and retracted even after the head of the imago has emerged, and the movement continues until the fly is free from the pupal case.

Eggs were found on all the grasses and cereals sown, but on hatching not all of the larvae survived, and although third-instar larvae were found in all cases, there were marked differences in the percentage of plants infested in the different Gramineae. From the number of larvae found and the number of flies hatched out from the various pots it was concluded that the cereals and grasses were preferred in the following order:

(1) *Lolium perenne.*
(2) *L. italicum.*
(3) Wheat.
(4) { Barley. / *Poa trivialis.* / *Dactylis glomerata.* }
(5) { *Cynosurus cristatus.* / Oats. }
(6) *Festuca rubra.*

(c) *Parasites*

Three species of parasites were bred; these were, *Phaenocarpa livida* Hal., *Chasmodon apterous* Nees, and *Stenomalus* sp. The percentage of parasitized puparia was not determined but the most frequent parasite was *Phaenocarpa livida.*

Of the *Stenomalus* sp. Dr Ferriere states *in litt.*: "This seems to be distinct from *S. muscarum* and is not in the British Museum collection."

III Structure

(*a*) *The third-instar larva*

(i) *Morphology.*

The third-instar larva is white and opaque, apodous and amphipneustic. It consists of a head, three thoracic and eight abdominal segments. It varies from 4·0 to 6·6 mm. in length and from 0·8 to 1·2 mm. in width in the widest part, which is that region from the third thoracic to the first abdominal segment. The tapering of the anterior end from this point depends upon the extent of retraction of the head within the first thoracic segment.

The *head* (Fig. 1) is small, rounded anteriorly and tapering posteriorly so that when retracted it fits into the first thoracic segment. When the larva is feeding the

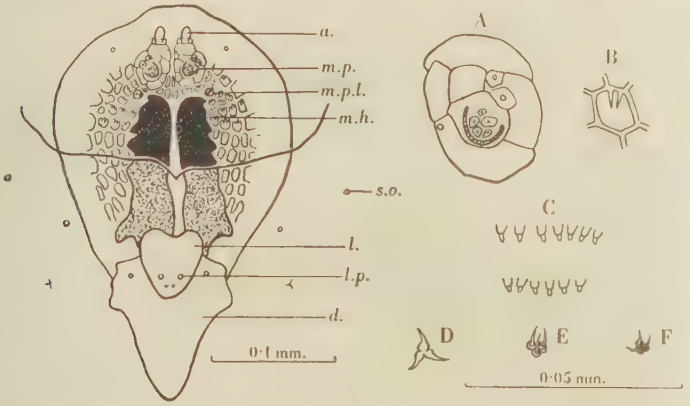

Fig. 1. Ventral view of head of third-instar larva of *G. tripunctata*. *a.* antenna; *m.p.* maxillary palp; *m.p.l.* sensory palp; *m.h.* mouth-hooks; *s.o.* prothoracic sensory organ; *l.* labium; *l.p.* labial palp; *d.* dentate sclerite. A, maxillary palp; B, chitinous teeth; C, thoracic chitinous denticles; D, E and F, vestigial legs of first, second and third thoracic segments respectively.

mouth-hooks are intermittently projected and retracted through the mouth opening which is on the ventral surface of the head, whilst the head itself is extended from and retracted into the first thoracic segment.

The *mouth* opens on the ventral surface of the head immediately beneath the mouth-hooks and between the oral lobes, and leads directly into the pharynx.

The *antennae* (*a.*) are conspicuous, one on either side on the anterior margin of the head. They are two-jointed, having an anterior elipsoidal joint articulating with, and having its base sunk into, the end of the second or basal joint.

The *maxillary palpi* (*m.p.* and *A.*) are situated in front of and below the antennae. Five minute sensory papillae are enclosed in an incomplete chitinous ring, the whole being slightly raised on a short wide basal palp. There are also two small papillae on the outer margin of each maxillary palp immediately anterior to the opening of the chitinous ring, and a pair of minute palpi, one on either side of the head on the inner

side near the maxillary palp. Other cephalic sense organs are a pair of small preoral papillae, one on either side below the maxillary palp, and two minute palpi one on each anterio-lateral margin of the head.

Ventrally the head is almost completely covered with numerous chitinous ridges enclosing polygonal spaces; a number of these ridges have minute chitinous teeth arising from their margins and directed posteriorly as shown in Fig. 1 B. Immediately posterior to the mouth on the ventral surface of the head is a lightly chitinized plate—the *labial plate* or *labium* (*l.*). This bears near the posterior margin two pairs of sensory papillae, the posterior pair being smaller than and placed immediately behind the anterior pair; these are the *labial palpi* (*l.p.*) said by Keilin (1915) to be present in all the cyclorraphous larvae he has examined.

The junction of the body segments is marked by incomplete lines of minute *chitinous denticles* (Fig. 1 C), the arrangement of which has been studied by Mesnil (1935).

Each thoracic segment bears on its ventral surface near its posterior margin a transverse series of six minute sensory organs (*s.o.*) which appear to be sunk in very shallow pits; it also bears on its ventral surface on either side near the ventral line, and immediately posterior to the above sensory organs, a *vestigial leg* (Fig. 1 D). This vestige consists of three minute bristles raised on short basal papillae.

(ii) *The cephalo-pharyngeal skeleton* (Fig. 2).

The *cephalo-pharyngeal skeleton* as compared with that of other acalypterate larvae, is thick and heavily sclerotized. It consists of a pair of *mandibular sclerites* or *mouth hooks* (*m.h.* 3) articulating with an intermediate or *hypostomal sclerite* (*h.*) which is attached to the large *pharyngeal sclerites* (*ph.*). The latter are fused above to a *median dorsal sclerite* (*d.s.*) and below is a median ventral lightly sclerotized plate, the *dentate sclerite* (*d.*).

The mouth hooks articulate with the hypostomal sclerite by means of two slender sclerotized rods. As in the majority of phytophagous larvae the mouth hooks are toothed and move in a median longitudinal plane. The hypostomal sclerites are H-shaped, the articulating rods of the mouth hooks fit into the anterior hollow of the H and the posterior rods of the latter are firmly attached to the pharyngeal sclerites. Each pharyngeal sclerite consists of a single anterior projection and a dorsal and ventral posterior projection. A long thin rod from the anterior projection extends over one of the posterior rods of the hypostomal sclerite. Dorsally the pharyngeal sclerites are fused with and connected by the dorsal sclerite and ventrally are continuous with the base of the pharynx. The dorsal sclerite is not so heavily sclerotized and in the third-instar larva it cannot be detached from the pharyngeal sclerites. The dentate sclerite viewed from the ventral surface is triangular in shape, and from the lateral surface is ≻ shaped, it is very lightly sclerotized and bears two minute prominences on the ventral surface near the anterior margin.

(iii) *The respiratory system.*

The third-instar larva is amphipneustic. The *anterior spiracles* (Fig. 3 A) are very large and prominently placed; they are situated on the dorso-lateral surface of the body near the posterior margin of the pro-thoracic segment. They are particularly broad and are flattened dorso-ventrally, each bearing thirteen to fifteen *digitate processes* (*d.*) which have small openings at their apices. Each opening is surrounded

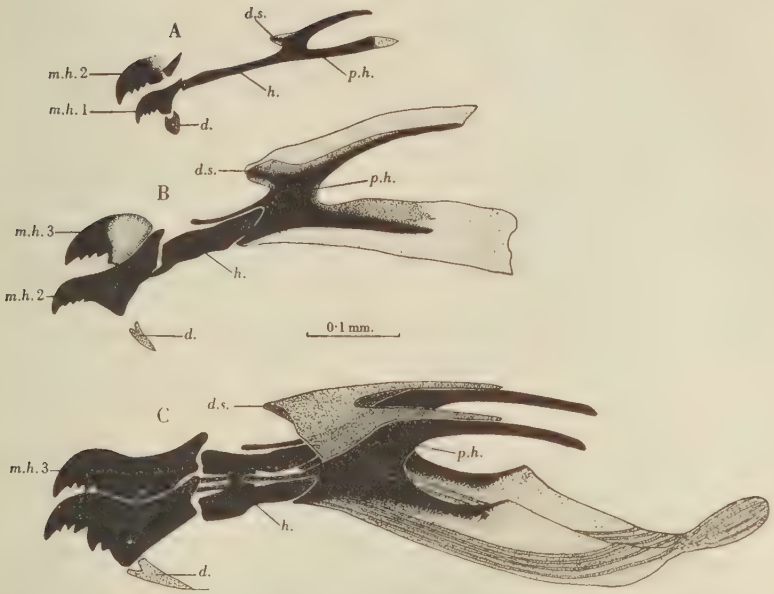

Fig. 2. Cephalo-pharyngeal skeletons of the three instars of larva of *G. tripunctata*. A, first instar; B, second instar; C, third instar. *m.h.*1, *m.h.*2, *m.h.*3, mouth-hooks of instars one, two and three respectively; *d.* dentate sclerite; *d.s.* dorsal sclerite; *h.* hypostomal sclerite; *p.h.* pharyngeal sclerite. (In A, *h.* = hypostomal region and *p.h.* = pharyngeal region.)

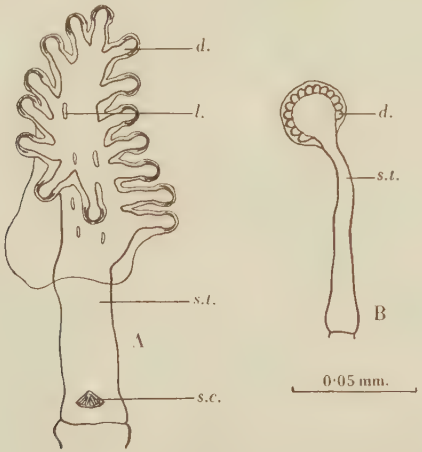

Fig. 3. A and B. Anterior spiracles of first- and second-instar larvae respectively of *G. tripunctata*. *d.* digitate process; *l.* lacuna; *s.t.* stigmatic trunk; *s.c.* stigmatic scar.

by a ring of chitin—the *peritreme*. A series of minute fissures are visible on the main stem of the spiracle which joins on to the short bulbous region of the main tracheal trunk by means of the *stigmatic trunk* or *felted chamber* (*s.t.*). There is a *stigmatic scar* (*s.c.*) on the felted chamber which marks the position at which the second instar larval spiracles became detached on moulting.

The *posterior spiracles* (Fig. 4 A) are situated dorso-laterally on the posterior end of the last abdominal segment; each is borne on the end of a short *stigmatic papilla*. The *stigmatic trunk* of each spiracle opens out on to the *stigmatic plate* (*s.p.*) through three short radiating branches, at the end of each of which is a small circular opening surrounded by a chitinous *peritreme* (*p.*). From the stigmatic plate arise four series of fine *branched chitinous hairs* (*b.h.*) which appear to support a thin membrane.

A main *dorso-lateral longitudinal trunk* (Figs. 4 and 5 B) extends along either side of the body to connect up the anterior with the posterior spiracles of each side.

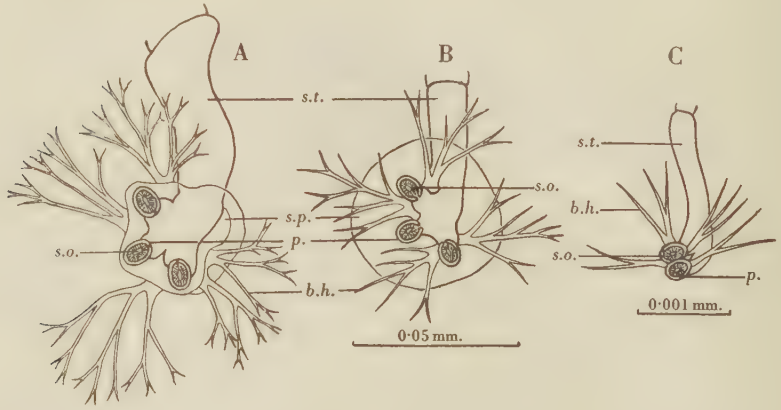

Fig. 4. A, B and C. Posterior spiracles of first, second and third instar larvae respectively of *G. tripunctata*. *b.h.* branched hairs; *p.* peritreme; *s.o.* stigmatic opening; *s.p.* stigmatic plate; *s.t.* stigmatic trunk.

A series of ten *dorsal transverse commissures* (1–10) unite one trunk with the other along the length of the body from the third thoracic to the eighth abdominal segment. The first and last commissures are thickened; the first which is in the anterior region of the third thoracic segment gives off two anteriorly directed branches, one from each side, to the dorsal region of the cephalo-pharyngeal sclerites; the second, in the posterior region of the third thoracic segment, loops forward over the first commissure to supply the posterior dorsal region of the pharyngeal muscles; the third arising in the anterior region of the first abdominal segment extends into the third thoracic segment; the second and third commissures have two unbranched tracheoles extending forward a short distance and arising one on either side near the mid-ventral line. The fourth, fifth, sixth, seventh, eighth and ninth commissures arise from the main trunks at the junction of the segments; the fourth arises at the junction of the first and second abdominal segment and others at each subsequent junction. These commissures loop slightly forward, each has two branched tracheae anteriorly, arising one on either side

near the mid-dorsal line, and two, one on either side directed posteriorly and arising a short distance from the point of junction of the commissures to the main tracheal trunk. The tenth transverse commissure unites the main tracheal trunks immediately behind the junction of the seventh and eighth abdominal segments.

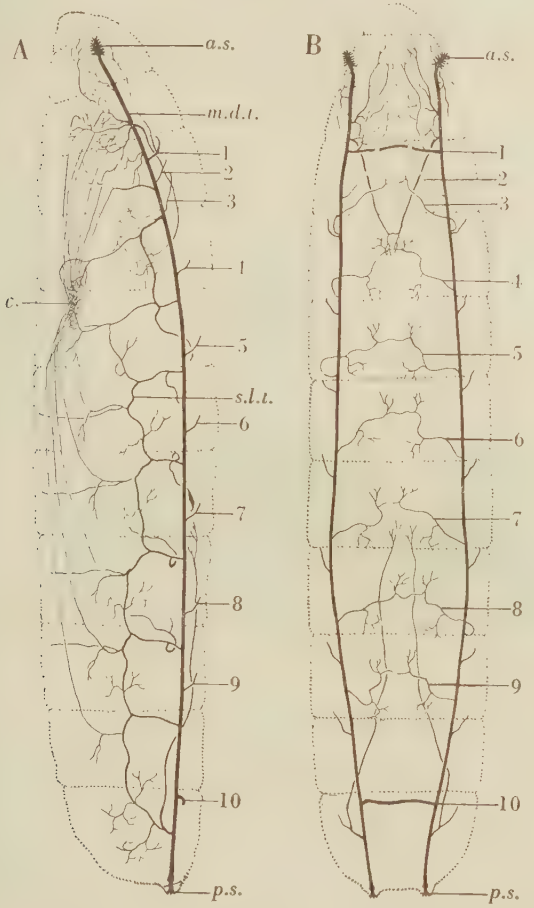

Fig. 5. Respiratory system of third-instar larva of *G. tripunctata*. A, lateral view; B, dorsal view. *a.s.* anterior spiracle; *m.d.t.* main dorsal tracheal trunk; 1–10, dorsal transverse commissures; *c.* concentration of tracheoles at "brain"; *s.l.t.* secondary longitudinal tracheal trunk; *p.s.* posterior spiracle.

The main trunks also give off into each segment a ventral branch. In the thoracic region these branches supply the pharyngeal mass and give rise to five long tracheoles on each side which run posteriorly to supply the brain. In the abdominal segments

the ventral branch subdivides giving an inwardly directed branch to the viscera and a branch which subdivides into a posterior and anterior branch which connect up with the corresponding branches of the adjacent segments to give a *secondary ventral longitudinal trunk* (*s.l.t.*). From this trunk in each of abdominal segments 1–7 a branch is given off which divides to give a branch to the ventral body wall and a branch which runs forward to supply the ventral ganglion (*c.*). The ventral longitudinal trunk also gives off small branches into each segment. The tracheae on either side run forward from the ventral longitudinal trunk to supply the ventral ganglion. After

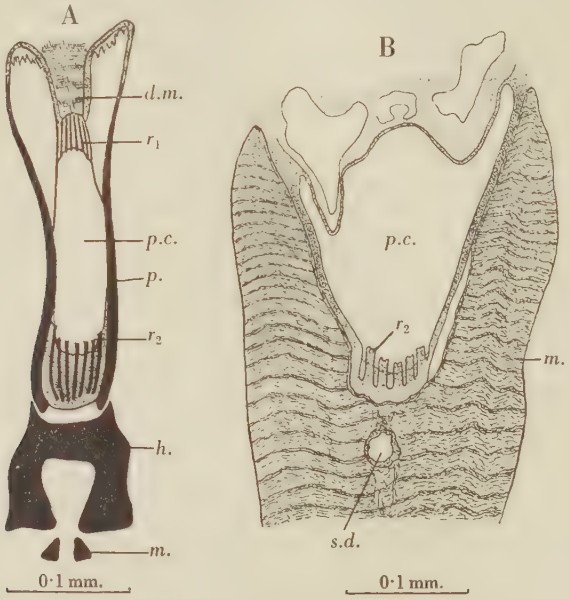

Fig. 6. Transverse sections of pharynx of third-instar larva of *G. tripunctata*. A, near anterior end; B, near posterior end. *d.m.* dilatory muscle; r_1, dorsal longitudinal ridges; *p.c.* pharyngeal cavity; *p.* pharyngeal sclerite; r_2, ventral longitudinal ridges; *h.* hypostomal sclerite; *m.* (in fig. A) mouth-hooks; *m.* (in fig. B) muscle; *s.d.* salivary duct.

entering the ganglion the tracheae subdivide and one tracheole from each branch on one side connects up inside the ganglion with a tracheole from a corresponding branch on the other side.

In the anterior region of the body other tracheae arise to supply the supra-oesophageal ganglion, their number and arrangement can be seen in Fig. 5 A. Six of these tracheae arise in the meso- and metathoracic segments, and one in the first abdominal segment.

(iv) *The pharynx.*

The *pharynx* (Fig. 6) which lies inside and along the base of the cephalo-pharyngeal skeleton, widens gradually from the mouth back to about three-quarters of its length

and then narrows again to join the oesophagus immediately behind the cephalo-pharyngeal skeleton. A series of transverse sections shows that the cavity inside the cephalo-pharyngeal skeleton is roughly oval in outline, and that the pharynx occurs at its trough-like base. Along the bottom of this trough there are six or seven longitudinal grooved ridges (r_2). Keilin (1915) has shown that the presence or absence of these ridges is dependent upon the feeding habits of the larva; that in saprophagous

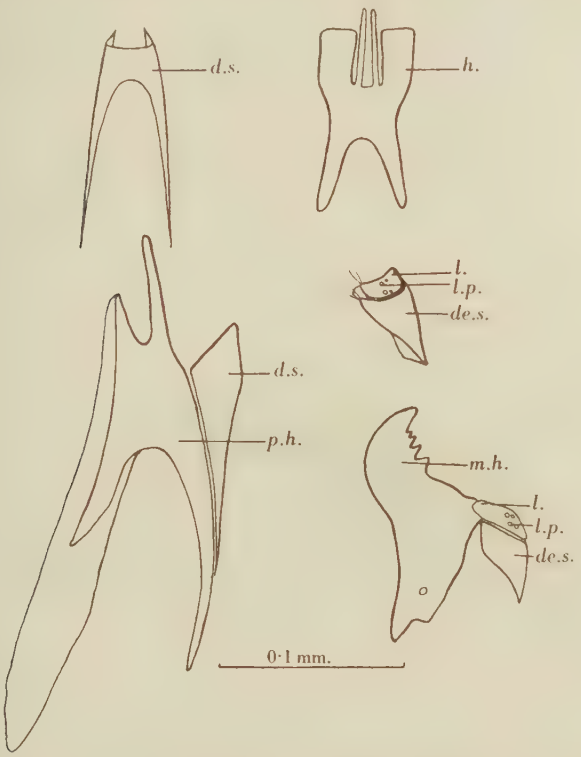

Fig. 7. Parts of cephalo-pharyngeal skeleton of second-instar larva of *G. tripunctata*. *d.s.* dorsal sclerite; *h.* hypostomal sclerite; *p.h.* pharyngeal sclerite; *l.* labium; *l.p.* labial palp; *de.s.* dentate sclerite; *m.h.* mouth-hook.

larvae they are generally well developed and that in phytophagous larvae they are generally absent. He has also shown that intermediate stages occur. This has been observed by Steel (1931) in the larva of *Oscinella frit* where the "arms" of the Y-shaped ridges are slightly longer than those of *Geomyza tripunctata*. Dorsally the pharynx is lined by a cuticular membrane folded into six small longitudinal ridges (r_1), U-shaped in transverse section. Above the cuticular membrane is a hypodermal layer which lines the cavity immediately above the pharynx and extends upwards as far as the insertions of the dilatory muscles. The latter are a pair of muscles which extend

longitudinally above the pharynx having their ventral insertions in its dorsal wall, and their dorsal insertions in the dorsal sclerite of the cephalo-pharyngeal skeleton.

(*b*) *The second-instar larva*

The second-instar larva is very similar in most respects to the third instar. It varies from 1·8 to 3·5 mm. in length and from 0·3 to 0·8 mm. in diameter in the region of the third thoracic segment.

The *head* and *cephalic sense organs* are miniatures of those of the third-instar larva. The vestigial thoracic legs (Fig. 1 E) and the thoracic sense organs are also homologous with, but smaller than, those of the third instar. The denticles on the thoracic segments are larger than those on the abdominal segments, those on the prothoracic segment being slightly larger than those on the meso- and metathoracic segments.

The number of component sclerites in the *cephalo-pharyngeal skeleton* (Fig. 7) is the same as in the third instar, all the sclerites are smaller but similar in shape. There is a pair of *mouth-hooks* (*m.h.*), a *hypostomal sclerite* (*h.*), a pair of *pharyngeal sclerites* (*ph.*), a *dorsal sclerite* (*d.s.*) and a *median ventral* or *dentate sclerite* (*de.s.*). Each mouth-hook differs slightly in shape from that of the third instar and is comparatively longer and less deep. It has a large apical tooth and four smaller ones which vary slightly in size and shape in different larvae. The teeth may be almost equal in size or the two posterior ones may be slightly larger.

The larva is amphipneustic and, except in the structure of the anterior spiracles, the respiratory system is almost identical with that of the third instar. Each *posterior spiracle* (Fig. 4 B) opens out through three *spiracular clefts* (*s.l.*) on a *stigmatic plate* (*s.p.*) at the end of a short papilla on the last abdominal segment. From the plate arise four series of *branched hairs* (*b.h.*) as in the third instar.

The *anterior spiracles* (Fig. 3 B) open out on the prothoracic segment and each has a comparatively long *stigmatic trunk* (*s.t.*), the number of spiracular clefts corresponds with the number in the subsequent third-instar larva. In this instar, however, the openings are situated at the ends of very short *digitate processes* (*d.*), each process being closely applied to its neighbour to form the major sector of a circle and the whole having the appearance of a transverse section through a rosette.

(*c*) *The first-instar larva*

The first-instar larva is very small, comparatively narrower and more translucent. Immediately after hatching it is about 0·9 mm. long and 0·17 mm. broad, but just before ecdysis it may attain a length of 1·8 mm. and a breadth of 0·3 mm.

The *head* (Fig. 8) is comparatively large, with a median ventral depression, the cephalic sense organs are homologous with those of the second and third instars. The *two-jointed antennae* (*a.*) are prominently placed on the anterior surface, the *maxillary palpi* (*m.p.*) are in a more ventral position and there are also a number of smaller *sensory papillae* (*s.p.*). The *labium* (*l.*) is lightly chitinized and bears a pair of minute papillae, the *labial palpi*, one on either side. There are no ridges on the ventral surface of the head similar to those described in the third instar. The *cephalic sensory organs* and *vestigial legs* are smaller but homologous with those of the second- and third-instar larvae. There are chitinous denticles at the junction of the segments as in the second- and third-instar larvae.

The *cephalo-pharyngeal skeleton* (Figs. 2 A and 8) is more simple than that of the second and third instars. Viewed from the lateral surface, only three sclerites can be distinguished—a *mouth-hook* (*m.h.*), a *median ventral* or *dentate sclerite* (*d.*) and a *pharyngeal sclerite* (*ph.*) termed by Keilin (1915) in the first-instar larva of *Pollenia rudis* "la pièce basilaire". The latter is not composed of two sclerites (the hypostomal sclerite and pharyngeal sclerite), as in the second and third instars, but consists of an anterior projection and two posterior projections. They are fused to one another only in the anterior dorsal region where there is a projection from either side to form a rudimentary *dorsal sclerite* (*d.s.*). Each mouth-hook has a large apical tooth and a series of small indentations. There is also an accessory sclerite (*a.s.*) at its base.

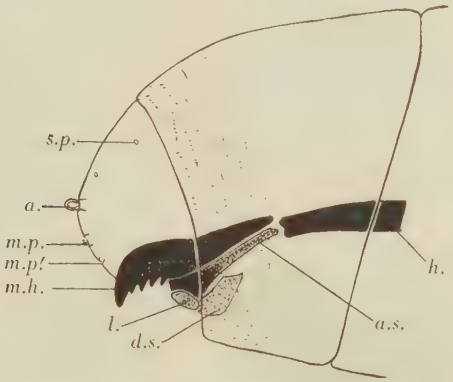

Fig. 8. Head of first-instar larva of *G. tripunctata*. *s.p.* sensory papilla; *a.* antenna; *m.p.* maxillary palp; *m.p.'* smaller palp; *m.h.* mouth-hook; *l.* labium; *d.s.* dentate sclerite; *a.s.* accessory sclerite; *h.* hypostomal region.

The first-instar larva is metapneustic as are the other first-instar larvae of cyclorraphous Diptera examined during the present investigation (Thomas, 1933, 1934). In the first instar larva of *Chlorops taeniopus*, however, Frew (1923) has observed that although hairs are present on the papillae of the last abdominal segment "these show no trace of stigmatic openings". Except for absence of anterior spiracles the general plan of the tracheal system in this instar is the same as that of the second and third-instar larvae. Each *posterior spiracle* (Fig. 4 C) has two *stigmatic openings* (*s.o.*), placed very near together at the end of a short *stigmatic papilla*; each opening is surrounded by a chitinous *peritreme* (*p.*) near which the *branched hairs* (*b.h.*) arise. There is a long *stigmatic trunk* (*s.t.*) which gives rise to a *main dorsal tracheal trunk*. In the region of the pro- and mesothoracic segments each trunk divides into tracheoles which supply the head and thoracic segments. There is a concentration of tracheoles in the region of the "brain" but this is not nearly as marked as in the second- and third-instar larvae.

(*d*) *The egg*

The egg (Fig. 9) varies in length from about 0·85 mm. when laid, to about 1·10 mm. immediately before hatching, and is about 0·2 mm. in diameter. It is fusiform in shape, slightly flattened on its ventral surface and more broadly rounded at the posterior than at the anterior end. It is white and glistening and its surface is marked by a series of longitudinal ridges and grooves most of which extend from one end of the egg to the other but occasional ridges end abruptly. The whole surface is covered by a series of minute papillae of thickened chorion, which are more numerous in the ridges than in the grooves. The anterior or *micropylar area* (*m.*) is considerably thickened and drawn out in the form of a plug of hardened chorion.

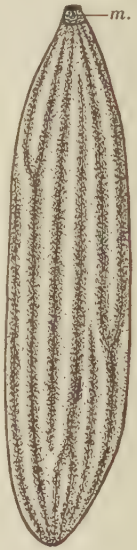

Fig. 9. Egg of *G. tripunctata*. *m.* micropyle.

(*e*) *The puparium*

The puparium (Fig. 10) is formed of the hardened integument of the third-instar larva. It is dark brown in colour and varies in length from 3·6 to 4·0 mm. with an average maximum breadth of 1·0 mm. It is approximately cylindrical in shape, the segments being homologous with those of the third instar. The thoracic segments are flattened on the dorsal surface which forms an angle of about 45° with the ventral surface. The larval head is completely retracted within the prothoracic segment, the larval spiracles (*a.s.*) remain protruding from the anterior end of the puparium. The posterior end is very wrinkled, the larval anus is present on the ventral surface of the last abdominal segment and the larval stigmatic papillae (*s.p.*) project from its posterior end.

The cephalo-pharyngeal skeleton of the larva remains inside the puparium fused into the ventral surface of the thoracic segments.

When the adult emerges, a slit is formed ventral to the edge of the sloping portion of the thoracic segments, this slit extends posteriorly as far as the anterior end of the first abdominal segment; in this region a horizontal slit occurs on either side, the two slits may meet dorsally when the flat portion of the thoracic segments becomes detached. The horizontal slit may also extend a considerable distance around the ventral half of the puparium so that the portion comprising the ventral half of the thoracic segments may be partially detached.

IV. Summary

In the field the chief larval host plants of *Geomyza tripunctata* Fall. are *Lolium perenne* and *L. italicum*; in the laboratory other grasses and wheat are readily attacked. The damage is similar to that caused by *Oscinella frit* L.; the larva feeds inside the grasses and kills the central shoot.

Adults emerge in March and April and there are two generations per annum; the species overwinters in the larval stage and pupation takes place inside the host plant near the base of the shoot.

The egg, the three larval instars, and the puparium are described.

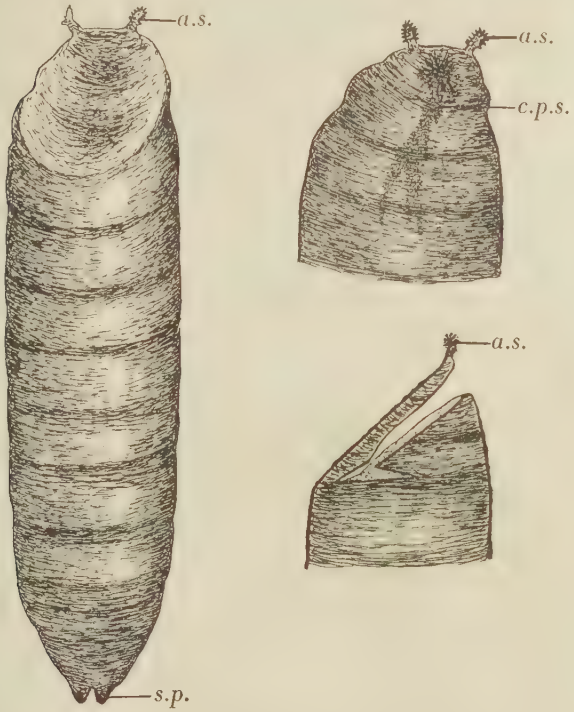

Fig. 10. Puparium of *G. tripunctata*. *a.s.* anterior spiracle (of larva); *c.p.s.* cephalo-pharyngeal skeleton; *s.p.* stigmatic papilla.

The writer is indebted to Dr H. W. Miles for help and advice during the early stages of the investigation when he was a Grisedale Scholar at the Victoria University, Manchester. He is also indebted to Mr F. R. Petherbridge for helpful criticism when the work was continued at the School of Agriculture, Cambridge.

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FIELD INVESTIGATIONS UPON THE CONTROL OF THE MUSTARD BEETLE, *PHAEDON COCHLEARIAE* F., ON WATERCRESS

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(With Plate IV)

CONTENTS

I. Introduction

During recent years, *Phaedon cochleariae* has been abnormally abundant on watercress in Glamorganshire, Monmouthshire, and many other parts of Britain. Attacks of great severity were reported during 1935 and 1936, and watercress was so extensively damaged in some instances that entire loss of crop resulted.

Most of the information hitherto available concerning control methods is contained in the Bulletin on Salad Crops and in Advisory Leaflet No. 157, published by the Ministry of Agriculture and Fisheries (1936*a*, *b*). The recommendation is made that in localities liable to infestation all watercourses and the sides of watercress beds, especially if no concrete work is employed, should be maintained in a clean condition, in order to decrease the number of places suitable for the hibernation of the adult beetles. For the purpose of dealing with attacks as they arise in summer, flooding of the affected beds with water for a few hours, where possible, is suggested since this would tend to drown the adult and larval stages

or sweep them away from the beds. There is also a brief reference in the leaflet to the use of derris or pyrethrum washes.

In 1931 pyrethrum and derris preparations were included by Thompson (1932) in preliminary field trials designed to discover a non-arsenical insecticide which would be sufficiently toxic to destroy the pest but which would not contaminate the watercress in any way or affect its market value. Both types of preparations were applied in the form of a spray on six occasions at intervals of a week, and the derris was also tested as a dust. After a preliminary trial the use of derris dust was, however, discontinued on account of the large amount required to produce satisfactory results and the high cost of the material. Both the pyrethrum and the derris washes were considered effective as a control measure though it was not found possible to estimate the actual percentage of beetles killed owing to the dense foliage of the watercress. The percentage of the active constituents present in the washes used in these trials is not stated.

Experience during the summer of 1934 showed that, apart from attention to sites which might provide winter shelter to the adult beetles, none of the measures advised for the control of *P. cochleariae* were practised by the commercial watercress growers in the counties of Glamorgan and Monmouth since these had been found unsatisfactory. Thus flooding of affected beds with the object of drowning or sweeping away the adults and larvae had been found only partially effective, probably because upon the rising of the water level a high percentage of the beetles invariably leave the watercress beds for the adjoining banks and are, in any case, capable of remaining afloat on the surface of water for a long time without being seriously affected. Further, watercress beds are generally low-lying with the result that the normal level of the water closely approximates the tops of the banks and the flow of water through the beds is usually too sluggish to wash away a large proportion of the insects. Pyrethrum had been tried by some growers as a spray fluid in the summer months, while others had relied for a time upon frequent applications of derris washes. The degree of control obtained by these methods had apparently been exceedingly inconstant and, on the whole, disappointing. This may perhaps be explained by the fact that both these insecticides suffer from the disadvantage of being variable and unstable substances and that, hitherto, no information has been available concerning the minimum potent concentration of the toxic constituent in the diluted solution of these products necessary to give the desired results.

The experiments described in the present communication were undertaken to investigate further the toxicity of derris and pyrethrum preparations to *P. cochleariae* on watercress, and to study the phytocidal effects of these products upon the plant itself.

II. General arrangement of the experiments

The experiments were carried out in 1935 at two different centres and in each case on watercress beds where attacks of great severity had occurred in the preceding two seasons. The results obtained at only one of these centres is discussed fully in the present paper, since the experiments at this centre were the more comprehensive and are essentially similar to those at the other centre. The experimental area consisted of 15 beds or plots (Pl. IV, fig. 1), each of which had an area of approximately 160 sq. yd. A group of three plots was taken as a unit for any one treatment. Four distinct preparations were tested and one group of plots retained as untreated controls. The treatments applied to the respective plots were as follows:

Plots 1*a*, 1*b*, 1*c*: Control (untreated).

Plots 2*a*, 2*b*, 2*c*: Derris wash with sulphonated lorol incorporated as a spreader, the wash being made up to contain 0·004 % rotenone and 0·05 % sulphonated lorol.

Plots 3*a*, 3*b*, 3*c*: Derris dust "A" having a rotenone content of 0·2 %.

Plots 4*a*, 4*b*, 4*c*: Derris dust "B" having a rotenone content of 0·5 %.

Plots 5*a*, 5*b*, 5*c*: Pyrethrum emulsion containing 0·01 % of pyrethrin 1.

The following diagram shows the arrangement of the plots together with the nature of the treatment applied to each plot:

Plan of experiment

2*a*	3*a*	1*a*	4*a*	5*a*	3*b*	2*b*	1*b*	5*b*	4*b*	2*c*	3*c*	1*c*	5*c*	4*c*

III. Application of the insecticides

By the end of June an enormous population of *P. cochleariae* had established itself upon the watercress beds, many of the plants bordering the banks being eaten to such an extent that little but the stems and the mid-ribs remained. Eggs, larvae and adults were present and as far as could be judged visually, the infestation was of uniform intensity over all the plots throughout the experimental area. Two applications of each

insecticide were given, the first on 24 June and the second on 5 July. The weather on each of these dates was very warm, sunny and calm except for an occasional slight breeze. Drift of the different preparations to neighbouring plots was avoided by the careful use of a hessian screen. No rain fell during the period 24 June to 10 July when the counts were made of the number of live beetles present on all the treated and control plots.

The machines used for the purpose were a pneumatic knapsack sprayer for the washes and a rotary hand blower for the dusts. Both spray fluids were applied at the same pressure and in such a way that the entire foliage was thoroughly wetted. The two dusts proved easy to handle and gave an excellent cover. Approximately equal amounts of each were utilized on the respective plots, though there was necessarily some variation between the plots even under the same treatment on account of slight differences in the size of the plants. In all cases sufficient dust was used to cover the whole of the foliage satisfactorily.

Application of all the insecticides on both occasions was done personally by the writer so as to ensure uniformity of treatment. The applications were aimed at killing the maximum number of the adults and larvae, and therefore all of them were made during the sunniest part of the day when the greatest number of beetles collected on the upper surface of the leaves.

IV. Estimation of results

Some 4 or 5 days after the second application of the insecticides, estimations were made of the actual number of live beetles present on both treated and control plots. The technique adopted consisted in counting all the live beetles that could be seen on all the plants within areas of 1 sq. ft., ten random areas being taken as the unit for the determination of the beetle population on any one plot. Each square foot area was defined by a wooden quadrate placed in position on the day prior to that on which the second application of the treatments was made.

Care was exercised when the counts were made to stand a short distance away from the area under examination and facing the sun so that no shadow was cast on the plants, since the beetles immediately drop and feign death when disturbed. Further, all the counts were conducted during the hottest hours around midday, the period when the beetles are most active and the majority feeding on the dorsal side of the leaves near the crown or the growing point of the plant.

The relative index figures of the beetle population per square foot on both treated and untreated plots are recorded in Table I.

V. Discussion of results

From an examination of the figures for the beetle population in Table I, it is evident that the degree of infestation was of a fairly uniform order over the area covered by the plots judging by the number of beetles present on the different control plots (Nos. 1*a*, 1*b*, 1*c*). A further striking feature is the very high population of beetles throughout the experimental area. The average number for the three control plots was 322 to the square foot or approximately 14 million per acre.

Table I

Relative index figure of the beetle population after treatment

Treatment	Index of plot	Number of beetles per 1 sq. ft.	Average % decrease of beetles
Control (untreated)	1*a*	311	
	1*b*	332	Nil
	1*c*	323	
Pyrethrum wash	2*a*	121	
	2*b*	131	62·1
	2*c*	114	
Derris wash	3*a*	81	
	3*b*	63	77·6
	3*c*	72	
Derris dust "A" (0·2% rotenone)	4*a*	37	
	4*b*	44	86·3
	4*c*	51	
Derris dust "B" (0·5% rotenone)	5*a*	10	
	5*b*	12	96·9
	5*c*	8	

The figures for the average percentage reduction in the beetle population, shown in the final column of Table I, indicate that all the insecticides tested had been used with decided advantage. The greatest reduction is found on the plots treated with derris dust of 0·5% rotenone content (plots 5*a* 5*b*, 5*c*). The average number of beetles on these three plots was 10 per sq. ft. compared with 322 for an equal area on the controls (plots 1*a*, 1*b*, 1*c*) showing a decrease in infestation due to this treatment averaging 96·9%. A reduction of such a heavy infestation to a figure in no instance greater than 3·7% (plot 5*b*) by dusting the watercress plants on two occasions is considered highly satisfactory from a practical standpoint. Immediately after the dust had been applied on both occasions, a large number of beetles and larvae were found floating on the surface of the water under the dusted plants, definitely affected

by the dust. Observations on the day following treatment showed that all the beetles and larvae were dead except for a few isolated adults which appeared entirely free from any deposits of the dust on their bodies. Moreover, the plants on the plots which received this treatment did not again become appreciably infested whereas on the controls the beetle population continued to develop and the plants were so badly attacked as to be quite worthless and unmarketable. The photograph on Pl. IV (fig. 2) was taken during the third week in July, that is, a fortnight after the second application of the insecticides, and shows the appearance of a typical plant on the left from one of the plots (plot 5*a*) dusted with derris of 0·5 % rotenone content and, on the right a plant from one of the controls (plot 1*a*). In order to safeguard against the movement of beetles to the treated plots, all the controls were dusted in the latter part of July with this derris preparation.

The next most efficacious insecticide was derris dust of 0·2 % rotenone content (plots 4*a*, 4*b*, 4*c*). The two applications of this dust had reduced the average number of beetles to 44 per sq. ft., a decrease of 86·3 % by comparison with the infestation on the untreated plots. When this dust was used it apparently destroyed all the beetles that were touched by it, but subsequent observations revealed that many of the supposedly dead beetles recovered some time afterwards.

The derris wash also showed a marked toxicity towards the beetles at the concentration tested (plots 3*a*, 3*b*, 3*c*). The intensity of infestation compared with that on the controls had been reduced by this treatment from an average of 322 to 72 beetles per sq. ft. The percentage mortality caused by this treatment was therefore at the rate of 77·6 compared with 86·3 and 96·9 for the two derris dusts. The limitation of the degree of control given by the derris wash was probably due at least in some measure to the low, dense foliage produced by the watercress plant and, in consequence, the impossibility of applying the spray in such a manner as to reach all the beetles and the larvae present on the undersides of the leaves. It was also found in later observations, as in the case of the derris dust of 0·2 % rotenone content, that many of the beetles which at first appeared dead were only temporarily paralysed.

The pyrethrum wash afforded some considerable protection against the beetle, but did not, in the circumstances described, prove of equal value to the derris preparations. The number of beetles found after treatment on the plots which received this insecticide (plots 2*a*, 2*b*, 2*c*) represented about a third of the infestation on the controls (plots 1*a*, 1*b*, 1*c*). Under the most favourable conditions, with very thorough and

careful application, the pyrethrum wash killed 62·1 % of the beetles, whereas the derris wash showed a toxicity of 77·6 %. No definite conclusions can be safely drawn as to the reasons for the failure of the pyrethrum wash to exert a control of the beetles at least equal to that derived from the use of the derris spray fluid but it may be pointed out that such failure might have been due to the difference detected in the wetting properties of the two emulsions rather than to any difference in the insecticidal potency of their respective toxic constituents. The spreading power of the derris preparation was definitely superior to that containing the pyrethrum extract.

VI. Difficulties involved in the general application of insecticides in commercial culture of watercress

Great practical difficulties are involved in applying insecticides in the field for the successful control of *P. cochleariae* on watercress plants. The use of arsenicals is open to serious objection on account of the difficulty in ensuring, even with thorough washing of the plants, that all traces of arsenic are removed before marketing the crop. The watercress grower is thus driven to other insecticides and from a consideration of the data presented in this paper it would seem that an efficient control can be secured by means of derris preparations which do not possess the poisonous properties of those containing arsenic. It must, however, be borne in mind that serious consequences may follow the indiscriminate use of derris or other insecticides containing rotenone since they are highly toxic to fish and other cold-blooded animals as well as to insects. They should on no account be used in circumstances in which there is any risk that the liquid or dust, even in very small quantities, might reach rivers, ponds or streams containing fish.

In addition to the danger of poisoning fish, it is realized that it would be unavoidable, in commercial practice, for some of the beetles sheltering on the undersides of the leaves or low down on the stems to escape the effects of such insecticides on account of the dense nature of the foliage produced by the watercress plant. For this reason several applications might become necessary in order to secure a satisfactory measure of control, the number being naturally dependent on the thoroughness of applications made and on the climatic conditions prevailing at the time of treatment. In bright sunny weather the beetles are exceedingly active, clustering in enormous numbers for feeding and mating purposes on the upper surface of the foliage in the region of the growing point or crown of the plant, whereas in dull weather they hide and comparatively

few are seen. Moreover, experience with contact insecticides in the control of other pests on different crops indicates that the penetrating and wetting properties of the wash would also have an important bearing on the number of applications necessary to give the desired results. Finally, in view of the overlapping nature of the life cycle and the fact that the insect is only vulnerable in the adult and larval stages, it follows that even the most successful treatment applied under the most favourable conditions would have to be repeated at definite intervals.

VII. Practical conclusions

In view of the observations recorded during 1936 from several centres in South Wales and Monmouthshire where watercress growers had tried out a schedule of direct control measures based upon a consideration of the results obtained from the 1935 field experiments described in this paper and from a study of the life history of the beetle, it is evident that attacks can for all practical purposes be suppressed by the judicious use of derris dusts containing not less than 0·2 % rotenone, if the conditions are such that there is no risk of poisoning fish. The dusts should be applied whenever possible in bright sunny weather, preferably during the hottest hours around midday, when the beetles are at the height of their activity and the majority on the upper surface of the foliage. This treatment when properly carried out will destroy both the adult and larval stages, but it is found desirable in commercial practice to examine the plants carefully some 24 hr. after application for the presence of beetles, and if numerous a further dusting should be given without delay. Applications of these dusts should be repeated after an interval of approximately 10 days in order to kill the larvae and adults which may have emerged since the first treatment, before the latter have attained maturity.

VIII. Summary

1. An account is given of field experiments in 1935 with pyrethrum and derris preparations for the control of *Phaedon cochleariae* F. on watercress plants.

2. Average infestations, judging by the beetle population on the untreated plots, were reduced by:

(*a*) Pyrethrum emulsion of 0·01 % content of pyrethrin I to 37·9 %.
(*b*) Derris wash of 0·004 % rotenone content to 22·4 %.
(*c*) Derris dust of 0·2 % rotenone content to 13·7 %.
(*d*) Derris dust of 0·5 % rotenone content to 3·1 %.

Fig. 1.

Fig. 2.

EDWARDS.—Field Investigations upon the Control of the Mustard Beetle, *Phaedon cochleariae* F., on Watercress (pp. 197–205)

3. The difficulties involved in the general application of insecticides for the successful control of the beetle in commercial culture of watercress are discussed.

4. A basis for controlling the beetle by means of direct measures is presented in the light of observations on extended trials by watercress growers in 1936, based upon a consideration of the results obtained from the 1935 field experiments and from a study of the life history of the insect.

Grateful acknowledgements are due to Dr H. Martin, of the Long Ashton Horticultural Station, for kindly determining the pyrethrin content of a sample of the pyrethrum preparation used in the 1935 field experiments described in this paper, and to the watercress growers in the counties of Glamorgan and Monmouth, particularly Mr W. Lewis, Llanmartin, Newport, for their helpful co-operation.

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EXPLANATION OF PLATE IV

Fig. 1. The general lay-out of the watercress beds at the principal centre involved in the field experiments upon the control of *Phaedon cochleariae* F. (see p. 202).

Fig. 2. Character of the damage caused by *Phaedon cochleariae* F. to watercress plants and the result of dusting with derris of a high rotenone content. Typical specimen, on the left from a plot dusted with derris containing 0·5% rotenone and, on the right from one of the controls (see p. 202).

(*Received* 18 *June* 1937)

NOTES

OBSERVATIONS ON PEAR SCAB (*VENTURIA PIRINA* ADERH.)

By W. F. CHEAL, D.I.C., N.D.A.

AND

W. A. R. DILLON WESTON, M.A., Ph.D.

School of Agriculture, Cambridge

(With Plate V)

In 1932 the writers commenced independent observations on the biology of the apple scab fungus (*Venturia inaequalis*) and the pear scab fungus (*Venturia pirina*) and, later, separate accounts of these were given (Cheal, 1933; Dillon Weston & Petherbridge, 1933). The following observations concern only pear scab and were made for the greater part in Cambridgeshire.

Observations on initial infection

One of us (Dillon Weston, 1933) had already determined by means of spore-trapping experiments that the expanding buds of Doyenne du Comice and Durondeau were infected by conidia from wood pustules before there was any apparent discharge of ascospores. It was of interest therefore to determine more precisely when this infection took place. Some pear trees in a private garden at Cambridge were kept under observation and it was noted that, on several of the scabbed twigs, the fungus stroma was exposed as early as February. Similar records have been made by Cheal (1933), Marsh (1933) and Salmon & Ware (1937).

In 1936 a pear tree which was wood susceptible was kept under daily observation and on occasions was examined during and after showers of rain. The small droplets which hung from some of the buds after the rainstorms were examined microscopically and found to contain conidia. At later stages of development when the buds were bursting, and also when the young rolled leaves were appearing, minute glass capillary tubes were inserted in these hanging drops and the water was examined. The samples which were taken contained conidia. It seemed clear, therefore, that many of these buds were literally bathed in spores and that when the water evaporated the spores were either left on the bud scales or, depending upon the degree of bud development, slightly drawn up amongst the young leaves and developing flowers. It was thought that if infection took place at this early stage it must follow that the under surfaces of the leaves and the calyces would first become infected. Observation showed that this happened. These records have a direct bearing on control measures and further support the conclusions arrived at by Marsh (1933) who states: "A longer programme of sprayings than for the apple is inevitable, and when pear trees are heavily infected with scab, much more than a single pre-blossom and a single post-blossom spraying is necessary."

The source of the inoculum which causes infection

In March 1933 three-year-old Fertility shoots were obtained from an orchard at Houghton in Huntingdonshire and it was observed that there were scab pustules on the one-year-old wood, none on the second, but that on the third-year wood the bark showed superficial cankers and had the appearance of having been badly attacked by scab. Since spores were noted here germination tests were made to see if they were viable and it was found that the germination percentage after 10 hr. was 1, as compared with 64 with the spores from the one-year-old wood. A similar observation was made on the variety Williams' Bon Chrétien but, in this case, no germination was recorded from spores on the three-year-old wood. It was then wrongly inferred that the spores which germinated from this three-year-old wood were spores which had been washed down from the viable pustules on the one-year-old wood.

One of us (Cheal, 1933) had drawn attention to the fact that Prof. E. S. Salmon had stated but not recorded that *Venturia pirina* persisted on two-year as well as one-year-old wood. As we could find no detailed observations on the production of viable spores from pustules on such wood the writers commenced a field investigation in the winter of 1936–7. Scab pustules were soon found on two-year-old wood but the pustules were cut off by cork barriers and flaked off easily when they were subjected to slight pressure, especially on young vigorous trees. On certain trees complete abscission of scab infected tissue was observed even on one-year-old wood.

It was in a seven acre plantation of twenty-year-old Conference trees at Tydd Gote that scab pustules producing viable spores on two-year-old wood were first found. It is of interest to record here that the fruit in this orchard had been badly blemished by scab for a number of years, since it is generally considered that this variety is one which is relatively resistant (Pl. V, fig. 1).

As it was established that scab pustules were present on two-year-old wood we made more detailed examination of older wood and found that in some cases this too was attacked. Pustules were noted on three-year-old wood on the Conference trees at Tydd Gote, on Pitmaston Duchesse in a private garden at West Malling, Kent, on Doyenne du Comice in a commercial plantation at Wilburton, and on an unknown variety in a private garden at Girton, Cambridge (Pl. V, figs. 2 and 3). The latter was a very old tree, suffered badly from scab and as far as is known had never been sprayed. Surface cankers on the wood were very apparent and, when these were further examined, it was seen that scab pustules were present on the edges of many of them and in some cases on four, five, six, seven and eight-year-old wood (Pl. V, fig. 4). We germinated the spores from some of these and found that they were viable. It would seem, therefore, that pustules of pear scab on wood older than one year are not uncommon, and it is inferred that in some cases, as yet not definitely explained, the fungus evades the cork barrier and so becomes perennial.

Control measures

It is suggested that these observations have a direct bearing on control measures and indicate further the importance of pre-blossom fungicide sprays and also their thorough application to older wood.

Acknowledgements

We are indebted to Prof. F. T. Brooks, F.R.S., The Botany School, Cambridge, for kindly confirming the presence of scab pustules on one of these older pear branches.

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EXPLANATION OF PLATE V

Fig. 1. Badly scabbed peduncle of Conference pear.

Fig. 2. Two-year-old branch of Doyenne du Comice showing scab pustules on the wood.

Fig. 3. Three-year-old branch of Doyenne du Comice showing scab pustules on the wood.

Fig. 4. Eight-year-old pear branch. Conidial pustules present at point indicated by the pins.

(*Received* 3 *August* 1937)

Fig. 1.

Fig. 2.

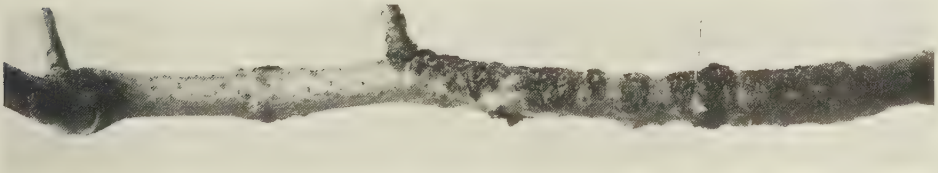

Fig. 3.

Fig. 4.

A FIELD OBSERVATION ON *OPHIOBOLUS GRAMINIS*

By W. A. R. DILLON WESTON

School of Agriculture, Cambridge

In the past four years several interesting cases of wheat or barley failures in Norfolk have been investigated. At or just prior to harvest, enquiries have been received concerning the cause of the thin stands, empty bleached ears, and prematurely ripened grain that are the features of such failures. The observations made suggest that, in many cases, the major predisposing factor leading to the condition is the taking of corn crops too frequently—or ill-advisedly—in the rotation, and that the chief cause of the trouble is the take-all fungus, *Ophiobolus graminis* and sometimes, in addition, wheat stem Sawfly, *Cephus pygmaeus* or Hessian fly, *Mayetiola destructor*. It is not the purpose here, however, to discuss the symptom complex of such failures or the pathogens concerned but to record an interesting case of a barley failure which was brought to my notice by Mr D. H. Findlay, of the Department of Agricultural Education, Norfolk. On a farm in 1933 a 14-acre field was sown with barley and in 1934, with sugar beet, the tops being ploughed in. Wheat was then taken on half of the field and oats on the other half and, in 1936, the whole field was drilled with barley. The manurial treatments per acre were as follows: 1933, 2 tons $CaCO_3$; 1934, farmyard manure and 4 cwt. superphosphate of lime; 1935, 3 cwt. superphosphate of lime; 1936, 1 cwt. sulphate of ammonia. The soil was light and sandy and of glacial origin. The *p*H of the top soil was 7·2; it contained 0·07% free $CaCO_3$ and the citric soluble P_2O_5 and K_2O were 0·018 and 0·006% respectively. These figures for citric soluble phosphate and potash are rather below the average for land of that type in that district and suggest a slightly subnormal level of fertility but no serious deficiency. In 1936, in the barley which followed that half of the field taken with wheat, 60% approximately of the tillers showed infection with *Ophiobolus* and 5% had been attacked by the Hessian fly. These facts were reflected in the yield, since the barley after the wheat was assessed at half a sack to the acre whereas, after the oats, it was estimated as yielding between 6 and 7 sacks per acre.

Garrett (1936) in a recent paper says: "*Ophiobolus graminis* can spread through the soil only along the roots of its host plant. A distinction can thus be made between two phases in the activity of the fungus, a parasitic or ascendant phase, in which the fungus is actively increasing on the roots, and a pseudo-saprophytic or declining phase, in which the fungus is merely persisting in dead host tissue. In the declining phase, the disappearance of the fungus from the soil must be hastened by the action of the soil saprophytes in actually decomposing its mycelium." It is of interest to speculate on the source of the infection on this particular field in Norfolk. That the disease was caused in 1936 by wind-borne ascospores from possible neighbouring sources is unlikely since, in that case, it would be reasonable to assume that the crop would be more or less uniformly infected. This was not the case. It may be, however, that in the previous year the half of the field sown with wheat was infected in this

way and that the pseudo-saprophytic or declining phase persisted on the dead roots until the barley was drilled.

An alternative possibility is that the 1933 barley crop was infected and that the declining phase lingered over in the soil to the autumn of 1935, when it was resuscitated by meeting wheat roots and so commenced again as ascendant parasitic phase. On the other half of the field, however, not wheat but oats was sown and the fungus was not able to survive on the roots of this host, consequently the barley that followed the oats was a successful crop, whereas the barley that followed the wheat was a failure.

The writer is grateful to Mr D. H. Findlay, the Department of Agricultural Education, Norfolk, for bringing this case to his notice and also to Mr F. Hanley, the School of Agriculture, Cambridge, for his analysis and comments on a soil sample that was taken from the field.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 8 October 1937, at the Imperial College of Science and Technology, London. The Chair was taken at 11.45 a.m. by Mr C. T. Gimingham (Vice-President) and at 2.30 p.m. by Dr J. Henderson Smith (President).

The following papers were read:

At the Morning Session:

I. The Wireworm Problem, with special reference to the north-west of England. By H. W. Miles, D.Sc.

At the Afternoon Session:

II. The Rook in the Rural Economy of the Midlands. By A. Roebuck, N.D.A.

III. The Food Habits of the Little Owl. By Miss A. Hibbert-Ware, M.B.O.U.

Summaries of these papers and of contributions by Mr W. R. S. Ladell and by Mr F. R. Petherbridge to the discussion on the wireworm problem follow.

I. THE WIREWORM PROBLEM

By H. W. Miles, D.Sc.
Victoria University of Manchester

The outlines of the wireworm problem are well known to most people interested in entomology and in agriculture and horticulture. Wireworms are the larvae of certain elaterid beetles called click beetles; they live in the soil for 4–7 years and feed on any available crop. The beetles are seldom seen in large numbers, but the wireworm population of the soil may reach hundreds of thousands per acre, and the loss caused by their feeding is probably greater than that caused by any other insect pest. At certain seasons the beetles are attracted to heaps of clover hay and the wireworms assemble in numbers to favoured foods. The usual habitat for the beetles and wireworms is grassland, and the older the grassland the denser the wireworm population. Serious injury by wireworms follows when grassland is broken up for arable cultivation. The insects seem to be most numerous on medium and light soils where intensive agriculture and market gardening are localized.

Various measures for the control of wireworms have been suggested, but the most effective methods involve a heavy expenditure that is only justified under market garden conditions and under glass where the crops have a high value. This Association

has already published accounts of the use of baits in conjunction with the application of calcium cyanide (Miles & Petherbridge), and of the reactions of wireworms to naphthalene (Tattersfield and Gimingham, etc.). The urgent need is for control measures suitable for use under general farming conditions, particularly for the protection of roots, potatoes and cereals. Long range research was begun in April this year at Warburton, Cheshire, and I propose to outline the conditions that make the wireworm problem of special importance in the north-west of England.

The North-West Advisory Province has a rainfall of 35–60 in. a year, which makes it suitable for dairy farming. Certain districts, particularly north Cheshire and south and west Lancashire, have sandy or peaty soils or mixtures of peat and sand, and in these districts intensive agriculture is followed since the proximity of large towns ensures a fairly good return for brassicas, potatoes, beet, carrots and peas. Potatoes and brassicas are also important to the dairy farmer since they are easily produced on his arable land and can be sold when prices are good or consumed at home when prices are poor. A sufficient acreage of grass is maintained by means of temporary leys, an essential feature of Lancashire and Cheshire dairy husbandry, and the usual rotation is oats, followed by potatoes (roots and brassicas), wheat, and seeds for one to three years. Leys are also a feature of the market growing areas; farmyard and stable manure is scarce so fertility is maintained by taking a crop of clover and rye grass and ploughing in the heavy aftermath. The rotation in these districts is oats or wheat followed by clover and rye grass, then potatoes and brassicas; or wheat followed by clover and rye grass, then potatoes and market garden crops, and finally oats. It is apparent, therefore, that throughout the entire region, leys are an essential part of the husbandry and potatoes a constituent of most rotations.

Leys are usually followed by wireworm infestation and where a rotation of four years or less is the practice there is a tendency for the wireworm population to increase steadily. Injury to potatoes receives most attention since attack is concentrated on the tubers, where it is easily seen. In a survey of the potato crop of 1936 it was found that half the farms in Cheshire suffered from wireworm infestation and that loss from wireworm injury to potatoes varied from 2*s.* 6*d.* to 40*s.* per ton. Losses on single farms varied from £24 to £528 and the total loss in the county reached almost £100,000. If to this is added the losses caused by wireworm attack on other crops some idea of the importance of wireworms in the North-West Advisory Province can be formed.

The chief difficulties facing the investigator are that leys are essential in the local systems of husbandry, and that the soils are good potato soils and no other crop can take the place of potatoes economically in the rotation.

Research work has been started along certain lines. Modifications in the rotation are being observed to see if injury to potatoes varies with the position of that crop in relation to the ley. Mechanical processes of cultivation, scuffling, and discing the stubble, which are said by farmers to give some relief from wireworm attack, are being observed in relation to wireworm injury, though it is difficult to see how they affect wireworms other than indirectly. The effect of the potato crop on the subsequent wireworm population is being studied since there is some evidence that numbers of insects are removed with the crop. The effect on the wireworm population of mustard ploughed in green is also under observation since this has been recommended as a treatment for wireworm infested land, but up to the present no direct effect on the insects has been noted. Observations are being made on the movements of wireworms

in the soil, and these indicate two main periods of activity at the surface: late April and early June, and early September to the end of October. Direct control measures with chemicals have not yet been attempted because sufficient biological and ecological data have not yet been collected.

In the discussion following Dr Miles' paper, Mr W. R. S. Ladell said that, at Rothamsted, the problem was being attacked under three main headings: (*a*) Field experiments, (*b*) Chemotropic responses, (*c*) Fumigation experiments in pots.

(*a*) The object has been to find out how far it was possible to test chemical control measures by a field technique similar to that used in fertilizer and varietal trials, and what was the minimum amount of soil sampling that must be done in order to obtain a reasonably accurate estimate of the wireworm population. The distribution of the wireworms in the field was very uneven, and there was no apparent relationship between the numbers present in any one location and any single soil factor such as *p*H.

Three field experiments using old grass land were described. Wireworm populations were ascertained in every case by sampling the soil before and after treatment. At first soil samples $9 \times 9 \times 5$ in. were taken, but with the introduction of the Ladell flotation technique giving a more complete recovery of the wireworms, it was found possible to reduce the size to $6 \times 6 \times 6$ in. Local control was used in the first two experiments and was found effective. The sampling errors were high, varying from 19%, when the numbers of wireworms were large, to 48%, when the numbers were small. In all cases the sampling error accounted for most of the experimental error. Although the errors were high they did not prevent us from detecting real differences between treatments. The first experiment was a 5×5 Latin square, size of plot 1/60th acre, six soil samples per plot. The second experiment was arranged in ten randomized blocks of three plots, size of plot 1/70th acre, four soil samples per plot. The third experiment consisted of eight blocks of six plots, size of plot 1/200th acre, two soil samples per plot.

The mean density of the wireworm population was 65,[1] 335 and 277 per sq. yd. respectively. Good results were obtained by the use of Seekay, Cymag, Chlorpicrin and Crude Naphthalene.

The possibility of using baits instead of direct counts on the soil was being explored. Both in the laboratory and field, cabbage leaf and stalk was found much more attractive than potato. No relationship was found between the number of wireworms obtained in the baits and the actual number in the soil. On plots with the most effective fumigants the baits contained the largest proportion of the real population, indicating a repellent effect of the fumigants into a more attractive environment.

(*b*) Three types of apparatus for finding out the chemotropic responses were described. Of vegetables tried, cabbages were the most attractive, especially cabbage stalk. Under some conditions germinating lettuce was preferred to germinating cabbage. Germinating barley was more attractive than oats or wheat. A few essential oils had been tried and, of these, mint was the best.

(*c*) Much attention has been paid to the design of pots for testing the value of fumigants against wireworms under conditions approximating as nearly as possible to those existing in the fields. Some progress has been made. Certain proprietary mixtures widely recommended were found to be useless. The best results were obtained with carbon disulphide, crude naphthalene and a sodium cyanide mixture, respectively.

[1] Without earlier instars.

Mr F. R. Petherbridge said that, at Cambridge, the work on wireworm has been chiefly in connexion with the sugar-beet crop. Attempts have been made to grow sugar-beet in fields with a high wireworm population. Experiments were designed to find out the value of extra seed rolling, drilling manure beneath the seeds, and drilling wheat between the rows.

In manurial experiments on barley carried out in 1934 by J. A. McMillan & F. Hanley, mixed fertilizers drilled with the seed gave a very much higher yield than did the same fertilizers broadcast in a field where a large number of wireworms were present. At four other centres where few wireworms were present and where these two methods of applying the manure were used, the crop differences were very much less.

In 1935 experiments drilling wheat between the rows of beet gave the largest increase in plant population.

In 1936 in a fen field near Southery (Norfolk) the beet crop was saved by drilling wheat between the rows and the plant population was further increased by extra seeding. The neighbouring plots where wheat was not sown between the rows had to be redrilled.

The following table shows the plant population per acre:

Wireworm experiments, 1937. *Beet population. After singling*, 29 *June*

	Row	Plants per row	Plants per acre
Heavy seeding 20 lb. per acre	1.....	967	22,531
	.. *W*		
	2.....	1032	
	.. *W*		
	3.....	1027	24,325
	.. *W*		
	4.....	1044	
	.. *W*		
	5.....	1073	
Normal seeding 15 lb. per acre	.. *W*		
	6.....	873	
	.. *W*		
	7.....	789	18,230
	.. *W*		
	8.....	686	
	.. *W*		
	9.....	630	14,679

W = Wheat between the rows.

Where 20 lb. of beet seed per acre was sown and wheat was sown between the rows at the rate of about 40 lb. per acre, the plant population after singling was almost perfect.

It is interesting to note that the outside rows of sugar-beet with wheat on one side only were very much better than the controls, and contained nearly as many plants as the rows with wheat on both sides.

II. THE ROOK IN THE RURAL ECONOMY OF THE MIDLANDS

By A. Roebuck, N.D.A.

Midland Agricultural College

The rook (*Corvus f. frugilegus*) is virtually part of the English country-side. Its large size, its characteristic cawings and its habit of appearing in flocks all the year round compel attention. It is essentially a bird of agricultural land. When feeding it almost invariably frequents farm lands at all times of the year and, as its diet is mixed, its economic position has been disputed from time immemorial.

Some years ago it was decided to attempt a survey of the rookeries in the Midlands, in order to find the number and distribution of the birds. It was decided also to study the kind of food eaten and the total weight consumed annually. Further problems studied have been the possible influence of migration, the competition of other species and the factors which operate as natural controls on the species.

The district surveyed has an area of 5305 sq. miles and comprises the counties of Nottinghamshire, Leicestershire, Rutland, Derbyshire and Lincolnshire. It includes a sea coast of about 100 miles on the East and on the West there are the mountains of Derbyshire rising to 2000 ft. The annual rainfall varies from under 25 in. for most of Lincolnshire to over 50 in. over a large part of Derbyshire.

For the purpose of the census the rookeries were located and the nests counted. By doubling the number of nests the number of nesting birds is obtained. Non-breeding immature birds are not included. The number of these birds varies in different rookeries.

The first survey was made in 1928–30 and this was repeated 4 years later.

The following are the principal data obtained from the first survey:

	Area (in sq. miles)	Number of rookeries	Nests	Birds
Nottinghamshire	843	182	6,501	13,002
Leicestershire	800	230	9,381	18,762
Rutland	152	49	2,340	4,680
Derbyshire	1009	240	10,620	21,240
Lincolnshire:				
Lindsey	1357	442	22,447	44,894
Kesteven	726	160	8,432	16,864
Holland	418	118	4,412	8,824
	5305	1421	64,133	128,266

Average size of a rookery 45·1 nests. 1 rookery to 3·7 sq. miles. 1 bird to 27·4 acres.

The rooks are by no means evenly distributed over these counties. Certain features of their distribution deserve special mention.

In Nottinghamshire they mostly keep to the low ground along the river valleys. There is a large area in the centre, roughly 200 sq. miles in extent, which has no rookeries. Jackdaws are the prevalent birds of the crow family in this area

A large portion of the eastern half of Leicestershire is grassland. On this part of the county the rooks are most abundant. In the grazing district there is one bird to 14·4 acres whereas there is only one bird to 31·3 acres in the rest of the county.

Derbyshire has about 260 sq. miles of land above 1000 ft. high. On this there are thirty rookeries of an average size of 55·5 nests and containing 3324 birds. This represents one bird to 50 acres. More than half of this area is treeless grouse moors with no rooks. On the remainder, therefore, the grazing land on the carboniferous limestone, rooks are as abundant as in the rest of the county, namely one bird to 21 acres. In contrast to the mountains of Derbyshire, Lincolnshire has a large area of fen where the tree tops are higher above the ground than the ground is above sea-level. There is one bird to 30 acres of land. The chalk wolds is the area richest in rooks, there being one bird to 10·5 acres. Although this is arable land there is an abundance of snails as a food reserve on the short leys, etc.

The second survey showed little substantial change in numbers or distribution as indicated in the following table:

	2nd survey		Lost. Sites abandoned	Gained. New sites
	Rookeries	Birds		
Nottinghamshire	180	12,150	29	27
Leicestershire	235	18,300	31	36
Rutland	46	4,136	8	5
Derbyshire	274	21,774	26	60
Lincolnshire:				
Lindsey	477	44,606	67	102
Kesteven	172	17,018	24	36
Holland	136	9,024	16	34
	1520	127,008	201	300

Their numbers had remained constant, but for various reasons 14% of the nesting sites had changed. These were mostly small rookeries and only about 4 % of the birds were involved. This should not be considered a change in 4 years but it is more nearly an annual change. In the intervening years many sites are known to have been occupied and abandoned which do not appear in either survey.

By the autumn the rookeries are deserted and any particular flock may join with a number of other flocks in the district and adjourn to some large wood for roosting at night. There are many exceptions. In some rookeries the birds will roost in trees alongside their nests all through the winter. The large roosts to which united flocks go during the winter are termed rook roosts, major rookeries or winter rookeries.

A survey of these roosts was made and the following numbers located: In Nottinghamshire 8, Leicestershire 13, Rutland 13, Derbyshire 34, Lindsey 58, Kesteven 23 and Holland 4, or 153 altogether.

As a rule the flocks leave the roosts each morning and return to their old feeding grounds for the day. During spells of bad weather, especially if foggy, they are often unable to get far from the roosts.

In order to obtain data on the food consumed by rooks birds were caught in traps and fed experimentally. These were mostly fully fledged young birds. The daily volume limit appears to be 140 c.c. whereas the daily weight has varied from 56 to 127·6 g. In estimations of stomach contents by volume, therefore, 51,100 c.c. must be considered per year, and by weight 56 lb. per bird per year.

The quantity of food consumed by rooks in this area per year thus approximates to :

Nottinghamshire	406·3 tons
Leicestershire	586·3 ,,
Rutland	146·3 ,,
Derbyshire	663·7 ,,
Lincolnshire:	
Lindsey	1402·9 ,,
Kesteven	527·0 ,,
Holland	275·7 ,,
	4008·2 tons

This food is not consumed evenly throughout the year. The surveys have dealt primarily with the nesting birds, that is when the population is at its lowest, but it is obvious that the population varies at different times of the year. One possible effect might have been migration. Numbers of rooks reach the Lincolnshire coast in the autumn but, contrary to what one might have expected, they move north or north-westwards. They appear to pass beyond the county. So far as the Midlands are concerned the writer can find no evidence that migration in any way affects the problem of the rook. With the advent of the nesting season our native population increases. It is impossible to do more than make a rough estimate of this increase in large rookeries. In some small rookeries observed, the population has been trebled by June, each pair having raised four young ones. On the other hand some rookeries fail to rear any at all. On the average the population begins to increase in March, reaches its highest in May, then there is a fall, at first rapid, then slower, until the normal population is again reached in the early autumn. During the months of rearing their young, examination of shot rooks has shown that the proportion of animal food in the diet is highest. It is during these months also that most of the bulk of the food is consumed. Allowing 5% for the non-breeding birds the total food consumed in the Midlands annually is about 4300 tons. Of this quantity 42% is consumed in April, May and June, and 66% is consumed in the period March to August during which time the young are fed by the parents.

The preference for the grazing lands of Leicestershire has been stressed. In addition almost all the rookeries in the area surveyed are alongside a grass paddock. The close proximity of a field of grass seems essential for their welfare. As animal food is necessary during the nesting season it is interesting to consider the distribution of such food on arable and grass land. A rook is not a scratching bird but has to use its beak to extract the smaller animals from the ground. It can only penetrate to a depth of 1½ in. Published data show that grassland is much richer in insect and other invertebrate life than arable land, especially at or near the surface of the ground. Moreover, on grass, on an average, 62% of this population can be reached by rooks, whereas only 27% can be reached on arable land. It will be seen, therefore, that grass is much more dependable for insect food than arable. It is also easily seen why rooks much prefer to follow a plough on arable land and why they rarely attempt to follow a seed drill.

The difficulties of interpreting data on the analysis of stomach contents are almost insuperable. Assuming a rook ate only one kind of food all the year round, it could consume

585,000 grains of wheat,
or 1,277,500 wireworms,
or 204,400 leatherjackets,
or 81,500 slugs and snails, etc.

The difficulty is that it eats some of each as well as many other things. Moreover, it readily adapts itself to almost any kind of food in an emergency. In the Midlands undoubtedly in the aggregate it confers inestimable benefits to farmers. Average conditions, however, do not exist anywhere. The work done during these surveys indicates that the question of possible damage done is purely local—in the immediate vicinity of the rookery.

III. THE FOOD HABITS OF THE LITTLE OWL (*CARINE NOCTUA* VIDALII)

By Miss A. Hibbert-Ware, M.B.O.U.

The conclusions as to the food habits of this bird are based on the results of the investigation into the nature of the food of the little owl, which was organized by the British Trust for Ornithology from February 1936 to July 1937. The investigation covered a wide field of observation. Over twenty regular observers sent food material to the writer, for analysis, at intervals, daily, twice weekly, weekly or fortnightly. Many others contributed by means of occasional consignments of food material and by first-hand evidence of their observations. In this way thirty-four counties and eighty-one districts were represented.

There were four sources from which evidence as to the nature of the food might be drawn:

(1) The faeces of the bird. The results from these are negligible since nothing but the merest traces of solid matter, e.g. a few rodent hairs, appeared in the faeces.

(2) The food castings or pellets, evacuated from the gizzard by way of the beak. Of these 2460 were analysed in one year, from February 1936. These contained not only the hard indigestible parts of the food, such as bones, fur, feathers and chitinous fragments but also, quite frequently, undigested soft matter such as portions of earthworms and entire insect larvae.

(3) The nest and so-called "larders". Of these seventy-eight were examined; the holes in almost every case were cleared to the base and the contents sent to the analyst. The "larders" contained chiefly the remains of prey taken to these holes for carving purposes before supplying it to the nestlings, e.g. wings of birds and unused portions of mammals. The nests contained superficially other parts of the same animals, e.g. tail quills, legs and contour feathers, portions of the pelts of mammals, etc. The large amount of debris in the nest (sometimes weighing several pounds) consisted chiefly of crushed pellets evacuated by the nestlings and sitting bird. Insect remains were a dominant feature of this debris.

(4) Gizzards of dead little owls. The contents of these sometimes gave valuable evidence of the nature of the last meal taken by the bird. Frequently however the gizzard was found to be empty or with mere traces of mammal hairs, etc., left over from the last pellet evacuation. This was the case with twenty-five of the fifty-one gizzards examined.

The food material obtained from the above three sources show conclusively that the little owl has a habitual diet, alike at all seasons, at all stages of growth and in all parts of the British Isles. This normal diet consists of invertebrates and small mammals. During the nesting season, from May to July, larger mammals and birds

are added in large enough numbers to be included as part of the regular diet, though not to the exclusion of the other items.

A marked feeding habit of the little owl is that it is a ground-feeder to a large extent. The invertebrates found in greatest abundance in the food remains are millipedes, woodlice, earthworms and particular kinds of insects, e.g. earwigs, carabid beetles, dung beetles, weevils and elaterid beetles. Most of these either have no wings or else seldom use them except under special conditions and the fact that their remains are, almost without exception, embedded in a matrix of soil, dung or moss, etc., shows that they were picked up from the ground. Cockchafers are extremely abundant in nest debris and field observers have watched little owls pick them up as they emerged from the puparia in the soil. The great predominance of eggs in the pellets containing *Tipula* (daddy-longlegs) makes it probable that the insects were taken in the act of egg-laying in the soil. In these pellets, obtained from eleven counties in large numbers, two only contained crushed *Tipula* remains without or with very few eggs, the rest were composed of eggs in a matrix of crushed *Tipula*. The field mice, voles, shrews, which form a considerable part of the diet throughout the year and the rats, rabbits (young), house-sparrows, starlings, blackbirds and thrushes which are added during the nesting season are either ground fauna or such as constantly frequent the ground. The normal food of the little owl consists therefore of common ground fauna. There is a very great numerical drop between the animals already named and those on the long list of animals taken occasionally or rarely, but these too are to a large extent procured from the ground.

An examination of the short list of animals which form the habitual diet shows that the little owl must be mainly crepuscular and nocturnal in its feeding habits. The invertebrates and mammals are almost entirely those that are active by night. The four birds commonly used as food from May to July are easily procured at evening and dawn. This inference that the little owl is not much of a day-feeder is also borne out by the following facts:

(1) The field observers have not been able to detect the bird in the act of serious hunting till evening. In two instances they have watched it picking up small objects by daylight, but every observer except one has named from 6.30 p.m. as the hour when hunting began. This observer noticed that near the end of the nesting season, the pair under his observation took to day-hunting. This may frequently happen but it has not been proved to be a habit.

(2) The empty condition of twenty-five of the gizzards examined points to the probability that the pellet, representing the food of the bird during 24 hr., is evacuated after the night's feeding before the bird roosts or becomes inert, and that no serious building-up of a fresh pellet usually begins during daytime. Thus, the habit of the little owl appears to be primarily that of a night-feeder. Daylight hunting is sometimes practised but does not constitute a habit.

Summing up, it can be said that the main feeding habits of the little owl have been shown, in the light of the recent investigation to be: (1) that it has a regular diet common to all districts and seasons, (2) that it feeds chiefly on ground fauna, (3) that it is largely crepuscular and nocturnal in its habits.

Certain food habits often ascribed to the little owl have been disproved by the investigation:

(1) It is frequently asserted that insect food is not used during the nesting season.

Not only, however, has the nest debris been found to be literally studded with clytra, heads and legs of beetles, but the gizzards of the few nestlings sent for examination all contained insect remains. One nestling of about 10 days contained in its gizzard a large fragment of a dor beetle and of a cockchafer. The nest tree can often be detected by means of the accumulation of insect fragments at its base.

(2) It is stated by others that the young are fed on delicate food obtained by raiding the nests of other birds. This investigation has shown that nest-raiding is a rare occurrence, and is not a habit of the little owl. The food of nestling little owls consists partly of larger prey than is used during the rest of the year, namely rats, young rabbits, starlings and blackbirds. Even the beetles used at this season are large species, chiefly cockchafers and dor beetles. There is no predilection shown for delicate food. The aim of the parents is quantity, not quality.

(3) It is further asserted that the young are fed largely on carrion beetles, chiefly burying beetles. It is true that the little owl picks up these beetles occasionally. Seventy-five burying beetles were found in seventy-eight nests and holes and in 2460 food castings. None were found in gizzards. It seems likely that burying beetles are occasionally attracted to the refuse dump in a "larder", also that the little owl turns over carrion lying about, as it does dung and so finds the beetles. But the paucity of the records shows that it is only occasionally that the little owl comes across carrion beetles during its hunting activities. Burying beetles, like stagbeetles, water beetles and many others must rank as occasional, not habitual food of the little owl.

REVIEWS

A Textbook of Plant Virus Diseases. By Kenneth M. Smith. Pp. x + 615 with a frontispiece and 101 illustrations. London: J. and A. Churchill, Ltd. 1937. 21*s*.

The study of plant virus diseases has shown an almost phenomenal development, since although plant viruses have been recognized since 1892, our knowledge is to all intents and purposes a post-war growth. It has been not only a rapid but, also, an interesting and somewhat untidy development. There has, for example, been no generally agreed opinion as to a technique for the examination of viruses, there has been wide divergence of view as to their classification, and there has been considerable controversy as to their nature. In consequence the literature which, in various languages is scattered in all sorts of journals, is more than usually full of speculative statements, observations, and experimental results the soundness of which is very difficult to estimate. For some little time the need has been urgent for a systematic treatment of the subject to-date, partly in order that both virus workers and general plant pathologists might get their bearings and, partly, that such a treatise should be compiled whilst this was still possible and before the rapidly accumulating mass of data became overwhelming. Further, with Stanley's discovery of the crystalline nature of, at all events, certain viruses it is possible that, in the next few years, the whole study of viruses and virus diseases may be revolutionized. At the close of a scientific period and before attention becomes diverted along the new avenues of exploration which open out, it is desirable that the results of investigations be surveyed critically and co-ordinated by one who has played an active role in the development of the subject. No one is more fitted to this task than Dr Kenneth M. Smith and, in its successful achievement, he has earned the gratitude of all interested in plant disease.

In writing a textbook of a subject of which no previous textbook exists the author must formulate some scheme of ordering and classifying the data. From time to time various methods for the classification of viruses and virus diseases have been proposed, all of them subject to criticism. The scheme adopted in the present volume is a compromise based on the method suggested by Johnson and it will undoubtedly receive criticism, but it has pragmatic justification in that it works. The author groups together all those viruses which are chiefly associated with a particular host plant. These basic viruses are then attached to the generic name of the host and numbered 1, 2, 3, etc., the strains of each virus being lettered alphabetically A, B, C, etc. Thus the numerous viruses chiefly associated with tobacco are grouped as *Nicotiana Viruses* 1 and 1 A–1 D, 2–12 and 12 A–12 B, and 13–15; those associated with the potato plant as *Solanum Viruses* 1–18. Composite viruses are treated similarly; thus the viruses of Rugose Mosaic of the potato, formerly *Potato Viruses* X and Y become *Solanum Viruses* 1 and 2, and the viruses causing Tomato Streak become *Nicotiana Virus* 1 and *Solanum Virus* 1. The total number of viruses included in the book falls into 52 groups each associated with a host name, thus *Delphinium Viruses* 1–2; *Pæonia Virus* 1; *Anemone Virus* 1; *Brassica Viruses* 1–4; and so on. By rigidly adhering to this scheme Dr Kenneth Smith has succeeded in resolving much of the chaos.

Further, throughout his book, the author follows a standard plan of treatment. The virus is first dealt with, its properties, mode of transmission, etc.; and then the diseases it causes, arranged according to the plant families, are described. The viruses as a whole are placed in the order of their plant hosts, Hutchinson's scheme of classification being followed. Thus under *Beta Virus* 1. Boncquet and Hartung, are first given a list of synonyms and the virus itself is then described under the headings:

resistance to various chemicals, alcohol and acetone; miscellaneous reagents; thermal death-point; effect of *p*H; dilution end-point; resistance to ageing; desiccation; filterability; attenuation and restoration of virulence; transmission. There follows an account of the differential hosts. The diseases caused by *Beta Virus* 1 in plants belonging to ten families are then described, and this section is followed by accounts of the geographical distribution of the virus and of methods for its control. Finally there is a list of the plants forming the host range of the virus. Where data are available for particular viruses or virus diseases notes are also given on electrophoresis, effects of radiation, serological reactions, particle size, crystallization, histopathology, cellular inclusions, carriers, varietal resistance, relation of diseases in various countries, and effect on yield. The balance of the author's consideration varies, naturally, with the state of knowledge concerning particular viruses or diseases. Thus nothing is known of the properties of *Lycopersicum Virus* 5 or *Lilium Virus* 1 whereas those of *Nicotiana Virus* 1 or *Lycopersicum Virus* 3 have received considerable study: with many viruses little is known of the host range whereas, with others such as *Callistephus Virus* 1 or *Cucumis Virus* 1, extensive lists of host plants can be given. The general recognition of these gaps in our knowledge, which become very evident owing to the author's mode of presentation, should stimulate considerable further research.

Throughout all this portion of his book the author's treatment of the subject is characterized by skilful selection of material and precision of statement and, within these seven chapters, he has succeeded in condensing an enormous amount of information and in keeping his discussion remarkably up-to-date. The work is a textbook and not an encyclopaedia of plant virus diseases and, although the vast majority of such diseases receive either description or mention, a few, such as virus diseases of the cactus *Epiphyllum truncatum*, of *Ipomoea batatas*, of *Dolichos biflorus* etc., are omitted whilst, of certain viruses, the author selects the more important diseases pointing out that, owing to their wide host range, it is not practicable to describe every disease produced by them.

Following the systematic consideration of the viruses and virus diseases is a lengthy chapter devoted to the insects, etc., concerned in their transmission. This, as one would expect from the foremost authority on the subject, is extraordinarily well done. A detailed description of each insect usually illustrated by a text-figure is given, together with an account of its life-history and habits, so far as these are known, ecology, food plants, viruses transmitted, and geographical distribution.

Ch. IX contains brief notes on a number of plant diseases which are suspected to be of virus causation but which require further study before they can be placed definitely in this category. Probably many readers of the book will wish to make extensive additions to this chapter but the author has been wise to err on the side of caution.

In the main part of the book the author begins with the viruses and then proceeds to the diseases caused by them: in the field one reverses the process, beginning with the plant showing symptoms of disease and working back to the virus. This practical difficulty is overcome by an appendix, in the first column of which the author lists the host plants alphabetically under their scientific names; in a second column the symptoms of specific diseases are described; and in a third column the names of the viruses causing the particular diseases are given, along with page references to their descriptions in the text. This appendix, which runs to 38 pages and gives the symptoms of 330 virus diseases occurring on 175 different host plants, is an extremely useful compilation.

Following two pages of addenda describing two viruses inadvertently omitted from the work, the book closes with a general index, an index of viruses arranged alphabetically by host names, and an index of authors. The general index does not include the names of those plant species which are merely listed in the text as forming the host range of any particular virus, but only those on which a disease is actually described. A full host index would have been a useful addition to the book.

Nicotiana Viruses 1–12 B occupy Ch. IV, whilst, for some unstated reason, *Nicotiana Viruses* 13–15 are carried over to Ch. V, which also contains *Lycopersicum*

Viruses 1–6, *Hyoscyamus Virus* 1 and *Datura Virus* 1. With the exception of Ch. IV, the bibliography of which is combined with that of Ch. V there is an excellent bibliography terminating each chapter. It is to be regretted, however, that the author has not adopted consistently in his citations the journal abbreviations as given in the World List of Scientific Periodicals.

In addition to the entomological figures of Ch. VIII, the book contains a coloured plate and 77 mostly full-page illustrations each with several photographs, delineating the symptoms of the virus diseases of the several hosts. The need, recognized almost from the beginning of virus study, of accurate photographic portrayal of host symptoms has enabled the author to make his book a very rogues' gallery of viruses and these illustrations, many of which appeared originally in the *Annals*, are a valuable feature of the book. The present volume in no sense supersedes the author's *Recent Advances in the Study of Plant Viruses* published in 1933. It is true that Chs. XII–XIV of the previous work are covered in a more up-to-date way by the specific accounts in the present book but, otherwise, the volumes are complementary and both are necessary to students of the subject.

By his experimental researches on viruses and virus diseases Dr Kenneth M. Smith had already made for himself an international reputation and this will be enhanced by his present volume. This work is not only an invaluable source book of information but the first real systematization and clarification of the science, and it will become the indispensable "Handbuch" of everyone interested in virus diseases.

WILLIAM B. BRIERLEY.

Economic Botany: A Textbook of Useful Plants and Plant Products. By A. F. Hill. Pp. x+592 with 225 text-figures. New York and London: McGraw-Hill Publishing Co. Ltd. 1937. 24*s*.

This is an excellent book. In an introductory chapter the importance and nature of plant products are discussed. The following eight chapters are devoted to industrial plants and plant products and deal respectively with fibres and fibre plants; forest products, wood and cork; forest resources; tanning and dye materials; rubber and other latex products; gums and resins; essential oils; fatty oils and waxes; and sugars, starches, and cellulose products. Two chapters on drug plants and drugs follow, dealing severally with medicinal plants and with fumitories and masticatories. The following seven chapters are devoted to food plants and deal, respectively, with the history and nature of food plants; the major cereals; the minor cereals and small grains; legumes and nuts; vegetables; fruits of temperate regions; and tropical fruits. The remaining two chapters concern food adjuncts, and deal with spices and other flavouring materials and with beverage plants and beverages. An appendix contains a systematic list, with both scientific and common names, of nearly 1000 species discussed in the text. There is a bibliography of 160 references arranged under five subject headings, and the book closes with a good index.

A book covering so wide a field must be selective. It includes the most important plants of America and of other parts of the world in so far as they enter into international commerce, but the author has not attempted to give the detailed morphology of the various species discussed or to consider too fully their agricultural and commercial aspects. Nevertheless, it contains a wealth of information on the botany, history and distribution of the plants, their industrial, medicinal and alimentary uses, the preparation and use of the plant products, and the economic importance of the plants and their products. Often, just sufficient information is given to make one wish to know a great deal more, and one turns to the bibliography, only to be slightly disappointed. The bibliography cites too many textbooks, and old editions and other works which have been superseded by more recent publications. Other than English and American references are almost entirely excluded, and no one of the great French books on economic botany is cited.

The book is up-to-date, well and interestingly written, and most pleasantly free from inaccuracies and misprints. Here and there one reads sentences which are a little surprising; e.g. "Plants have been and still are responsible for many of the social ills that beset mankind", "Perhaps the chief social problem for which plants are responsible is the narcotic drug habit and the illicit trade that has grown up around it", "the African natives...cannot be taught proper methods of tapping", "in the past the (Chinese) nation as a whole has shown in its mental and physical characteristics the effects of the opium habit", and so forth.

The author states in his preface: "Even though the value of including a considerable amount of economic material in a beginning course in botany may be recognized, the limitations of time or various curriculum requirements usually render such a procedure impracticable. It should be possible, however, to offer at least a half-year devoted to economic plants as a supplement to the usual first year's work." Coming from a member of the botanical staff at Harvard this is interesting and, if his point of view is at all widely held by American University teachers of botany, they must differ considerably from most of their English confrères. A difficulty hitherto, suggested as partly responsible for the active or passive opposition to the inclusion of economic botany in the ordinary botanical curriculum, has been the absence of easily available English textbooks on the subject. With the publication of the present work, the books by Stanford (*Economic Plants*, 1934; *General and Economic Botany*, 1937), the excellent little book by Good (*Plants and Human Economics*, 1933) and older books by Robbins and Ramaley, Barrett, Clute, Kraemer, and others, this difficulty can no longer be made an excuse for a situation which is due primarily to sheer conservatism and mental inertia.

Dr Hill has written an exceedingly useful book which should be in every botanical library and in constant demand by teachers and students alike.

WILLIAM B. BRIERLEY.

Practical Plant Breeding. By W. J. C. Lawrence. Pp. 155 with 34 illustrations. London: Allen and Unwin Ltd. 1937. 5*s*. 6*d*.

This little book by Mr Lawrence, Curator of the John Innes Horticultural Institution, is the best introduction I know to plant genetics. The author introduces his subject by describing the structure of flowers and the processes of pollination and fertilization, and follows this by a clear and practical account of the technique of breeding. Two chapters deal with the laws and mechanism of inheritance, and three remaining chapters with sterility, and the methods and results of plant improvement. The book opens with an appreciative foreword by Sir Daniel Hall, and closes with a short list of books for further study and an index.

The book is very clearly written and the author has been unusually successful in condensing and translating into simple language a mass of technical research in this difficult field. Indeed, considering its small size, the book covers all the main issues of the subject in an extraordinarily efficient way. It is packed with well chosen examples and is well illustrated.

In numerous ways Mr Lawrence has produced just the book that many of us have been hoping for; a small cheap book, understandable, accurate and up-to-date, giving just the right amount of necessary detail and yet maintaining a broad and suggestive outlook. It is ideal for amateur gardeners and nurserymen or for students of horticulture.

The only pity is that the book is purely horticultural since, in many institutions, students of agricultural botany and of horticulture combine for a short course in plant genetics. If, in a second edition, Mr Lawrence could include examples from agricultural crop plants so that the book would also appeal to students of agricultural botany, its size would not be greatly increased but its value would be doubled.

WILLIAM B. BRIERLEY.

British Stem- and Leaf-Fungi (*Coelomycetes*). Vol. II. *Sphaeropsidales and Melanconiales*. By W. B. Grove. Pp. ix + 407, with 102 text-figures. Cambridge: University Press. 1937. 21*s*.

Vol. I of Mr Grove's book which dealt with the *Sphaeropsidales—Hyalosporae, Hyalodidymae, Hyalophragmiae* and *Scolecosporae*, received notice in the *Annals*, **23**, 3, 1936. The second volume, which has now been published, completes the *Sphaeropsidales—Phaeosporae, Phaeodidymae, Phaeophragmiae, Dictyosporae*, the *Nectrioideae, Excipulaceae* and *Leptostromataceae*, and includes the *Melanconiales*. The general viewpoints and methods of treatment exemplified in Vol. I are continued throughout Vol. II. There is the same meticulous accuracy of nomenclature and diagnostic detail and the same pungency in Mr Grove's comments, many species erected particularly by Cooke or Massee, being criticized in no uncertain language.

Concluding the systematic treatment proper are three pages of addenda to Vol. I, and four pages of diagnoses of new genera and species. There follow an epilogue and a poem by Mr Grove both of which are entirely out of place in a book of this character. The book closes with indexes of Ascomycetes, of host plants, and of binomial names, an additional note on *Diplodia*, and a list of authorities with their correct abbreviations.

In his epilogue Mr Grove mentions that he was born in the year 1848, and the production of such a work as this at the age of 89 is an extraordinary achievement. Mycologists will not only felicitate him upon the attainment of so ripe an age but offer to him their gratitude for the splendid results of his labours.

WILLIAM B. BRIERLEY.

Plant Ecology. By Hilda Drabble. Pp. 142, with 12 Plates. London: Edward Arnold and Co. 1937. 7*s*. 6*d*.

A good elementary introduction to ecology. The first eight chapters deal with the mode of life of plants as individuals, and with ecological terms and concepts, and the remaining eighteen chapters with plant communities in relation to their habitats and to each other. There are a brief epilogue containing hints on study, a short bibliography of books and papers for further study, forty-five text-questions arranged by chapters, an index to plant names, and a general index. The book contains an Errata slip of six items which needs to be greatly extended. The photographic illustrations are excellent.

WILLIAM B. BRIERLEY.

The Properties and Functions of Membranes, Natural and Artificial. Pp. 911–1151. London: Gurney and Jackson. 1937. 12*s*. 6*d*.

This volume contains the papers and discussions of the Fifth Colloid Meeting of the Faraday Society held in April 1937. It is reprinted from the *Transactions of the Faraday Society* with the original pagination. The subject was discussed under the following heads: Part I. Natural Cell Membranes: (*a*) General—structure, permeability, membrane potential, anaesthesia; (*b*) Special—red cells, fish gills and egg membranes, plant cells, bioelectric phenomena; (*c*) narcosis. Part II. Artificial Membranes. There are fifteen papers and discussions in Part I and nine in Part II.

Many of the papers, although fascinating from a more theoretical standpoint, are of perhaps rather remote interest to applied biologists. Some, however, have direct bearing upon research in various fields of applied biology, and the attention of Virus workers particularly may be drawn to the paper by W. J. Elford on "Principles Governing the Preparation of Membranes having Graded Porosities. The Properties of 'Gradocol' Membranes as Ultra-filters."

The papers are interesting but very condensed statements of the position to-date of the various problems discussed, and the volume forms a splendid cross-section of this extraordinarily wide and difficult field.

WILLIAM B. BRIERLEY.

Perspectives in Biochemistry. Edited by JOSEPH NEEDHAM and DAVID E. GREEN. Pp. viii+361, 5 plates and a Frontispiece. Cambridge: University Press. 1937. 15*s*.

No person stands higher in the esteem of the scientific world than Sir Frederick Gowland Hopkins, and few have had so great an influence on their science either through their own researches or through the men and women who have gathered inspiration at their hands. It was therefore a singularly happy thought of his past and present students to celebrate his seventy-fifth birthday by writing and presenting to him this book of essays. It is a fine tribute paid to a great man.

Reflecting the wide interests of the founder and head of the Cambridge Biochemical Laboratory the essays touch on many aspects of the science of life with all its great bearing on human welfare. Physiology and zoology, embryology and genetics, medicine, bacteriology, and nutrition all pay homage, and essays on "The Biochemistry of the Individual" or "The Meaningless of the Terms Life and Living" join hands with essays on "The Economy of the Bacterial Cell", "The Chemical Regulation of Insect Growth", "The Biochemistry of Flower Colour Variation", "Biochemistry and the Pathogenic Viruses", and numerous other problems.

The aim of the writers has been to indicate the most promising lines of advance in the various fields which they survey, and while maintaining a due standard of criticism, to speculate a little on the likely paths of future thought and discovery. Each essay deals concisely with a particular topic of which its author has special knowledge and concludes with a bibliography. The essays are well written and interesting to read and, where all are so good, it is impossible to select individual essays for mention. One may, however, be forgiven for quoting a short passage from Prof. Marrack's essay on "The Social Implications of Biochemistry" since this shows well the potentialities of Sir Frederick's life-work. "We are no longer dealing with vague principles but with chemical compounds, whose physiological activity may be correlated with physical and chemical properties. As the sense of power over nature which this knowledge gives spreads out from the specialist to the general public, men will gather confidence to abandon traditional beliefs and inhibitions and to shape a society in which all the possible resources of science and production are used for the good of all men."

WILLIAM B. BRIERLEY.